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THE SYNTHESIS AND THE CHARACTERIZATION OF MONOMETHYL- AND DIMETHYLINDOLES[1]

BY LÉO MARION[2] AND CATHARINE WOOLSEY OLDFIELD[2]

Abstract

All the seven monomethylindoles and 18 of the 21 possible dimethylindoles have been synthesized by unambiguous methods. Eleven were prepared by the Fischer ring closure applied to the appropriate phenylhydrazone, five of which through the appropriate phenylhydrazone of ethyl pyruvate. The rest were prepared by the Tyson ring closure. The picrates of each indole and the styphnates of all but three were prepared and their properties recorded. The three unrecorded styphnates (those of 1,6-, 5,6-, and 5,7-dimethylindole) could not be isolated without decomposition although they seemed to exist in solution.

Introduction

The degradation of alkaloids belonging to the indole group often gives rise to substituted indoles, usually monomethyl- or dimethylindoles. However, many of the dimethyl- and even of the monomethylindoles have never been reported and it is often not possible to identify such products. A case in point is the isolation among the degradation products of yohimbine (3) of a dimethylindole that has not yet been identified. Until the recent discovery by Tyson (23, 24) that potassium alcoholates are excellent agents for the formation of indoles from *o*-formotoluidides, the unambiguous synthesis of certain dimethyl- and monomethylindoles, i.e., those substituted in either the 4- or the 6-position, was not possible.

The seven monomethylindoles and 18 of the 21 dimethylindoles have now been synthesized unambiguously and their picrates and styphnates have been prepared. Each solid indole was recrystallized until the melting point was constant and, when the indole was an oil at room temperature, it was distilled twice and a middle fraction taken. Many of the indoles deteriorate on keeping unless they are sealed in evacuated tubes. However, the picrates are stable. All the picrates were recrystallized to their ultimate melting points and the styphnates at least once. The melting points of the latter are, therefore, not quite as accurate as those of the former but are sufficiently so for purposes of identification. All the recorded melting points of the picrates and styphnates were taken by immersing the substance in the bath about 20° C. below the melting point in order to avoid prolonged heating. Both the

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picric acid and the styphnic acid used in the preparation of these derivatives were purified by recrystallization from hot water. The properties of the indoles, their picrates, and styphnates are listed in Table I. An examination of this table reveals that any monomethyl- or dimethylindole can be identified from its properties and the melting points of its two derivatives.

TABLE I

MELTING POINTS OF MONOMETHYL- AND DIMETHYLINDOLE DERIVATIVES

Indoles, and their melting points, ° C.		Picrate, m.p., ° C.	Styphnate, m.p., ° C.
1-Methyl		151	158
2-Methyl	62	139	147
3-Methyl	96.5	182	168
4-Methyl, ††		194	170
5-Methyl	59.5	161.5	172
6-Methyl, ††		161.5	160
7-Methyl, ††	85	177.5	154
1,2-Dimethyl, †	56	125	153
1,3-Dimethyl		145	133
1,4-Dimethyl, ††		187	158
1,5-Dimethyl, †		151	144
1,6-Dimethyl, ††		148	*
1,7-Dimethyl, ††	78	154.5	150
2,3-Dimethyl	108	159	159
2,4-Dimethyl		164.5	167
2,5-Dimethyl	115	159	149
2,6-Dimethyl, ††	88.5	138.5	146
2,7-Dimethyl, ††		156.5	149
3,5-Dimethyl	75	177	184
3,7-Dimethyl	64	165	152
4,6-Dimethyl, ††		169	168.5
4,7-Dimethyl, †	102	177	173
5,6-Dimethyl, ††	64	154.5	*
5,7-Dimethyl, ††		183	*
6,7-Dimethyl, ††	70	164	162

† *Indole previously prepared but picrate not made.*

†† *Indole not previously known.*

* *These styphnates form in solution but are dissociated as soon as an attempt is made to isolate them.*

The elementary micro-analysis for nitrogen of the picrates sometimes offers difficulties but this element can then be determined by the micro-Kjehldahl method following a preliminary treatment with hydriodic acid (7).

It should be pointed out that mixed indole picrates are sometimes very difficult to separate and in such cases it is advisable to regenerate the indole, distil, and reject end fractions. Admixture of an indole picrate with another causes a depression in melting point of the order of 10° to 20° C.

The Syntheses

Although the classical Fischer indole synthesis (5) was extremely useful for the preparation of the indoles it was not always applicable, since when it is applied to *m*-substituted phenylhydrazones ring closure can take place in two different ways and give rise to a mixture of indoles. In almost all such cases, however, it was found that the method discovered by Tyson (23, 24) could be used successfully. Whenever this method was applicable it was found to be less tedious than Fischer's synthesis and generally gave better yields. The Tyson method also has its limitations and can give rise to mixtures as, for instance, when applied to 2,3,6-trimethylformanilide. The product would obviously consist of a mixture of 4,7- and 6,7-dimethylindoles. These two indoles were conveniently prepared by the Fischer synthesis.

7-Methylindole was prepared by the Fischer synthesis and although 1-, 2-, and 3-methylindoles were prepared by that method, they were also synthesized by the Tyson method (15) as well as were the remaining monomethylindoles.

With one exception all the dimethylindoles having one methyl group in position 1 were prepared from the appropriate formotoluidide by the Tyson method, which has been shown to be applicable to the formation of 1-substituted indoles (15). The Fischer synthesis was used for the preparation of 1,7-dimethylindole. It should be noted, however, that the purification of 1-substituted indoles prepared by the Tyson method was difficult because of the presence of small quantities of impurities. These impurities might, possibly, be 3-substituted homologues of the indole (21).

All the 2-substituted indoles were synthesized by the Fischer method except 2,4- and 2,6-dimethylindoles, which were obtainable readily and without ambiguity by the Tyson method. 2,3-Dimethylindole is also accessible by the latter method (15).

The dimethylindoles having substituents in positions 3,5-, 3,7-, 4,7-, and 6,7- were synthesized via the Fischer synthesis while the remaining dimethylindoles reported were obtained by Tyson's method.

Three dimethylindoles are still lacking to complete all the possible isomers. They are 3,4-, 3,6-, and 4,5-dimethylindoles. Their synthesis is under way and will be reported later.

It seemed unnecessary to give all the references relevant to this work and, therefore, only those deemed essential have been listed at the end of this paper.

Experimental

When the indole was a solid at room temperature it was recrystallized from a mixture of absolute ether and purified pentane until there was no further increase in melting point.

The picrates were prepared by adding a solution of a slight excess of the indole in ether to a solution of picric acid in ether containing a little methanol. The picrate was filtered off and recrystallized either from ether or from a

mixture of ether and methanol. There was, generally, no appreciable increase of the melting points of the picrates on recrystallization.

The styphnates were prepared by the addition of a methanolic solution of styphnic acid to an ether solution of the indole. They were recrystallized once from concentrated solutions in methanol. Although some styphnates could be recrystallized from ether, this was not always possible and in some cases the ether solutions deposited styphnic acid. In such cases, the addition of a little methanol prevented the compound from dissociating, and it crystallized. There were, however, three exceptions, i.e., the styphnates of 1,6-, 5,6-, and 5,7-dimethylindoles, which it has not been possible to isolate although the colour of the solutions indicated the existence of the styphnates in solution.

The Fischer Syntheses

This synthesis involves ring closure in the appropriate phenylhydrazone by fusion with zinc chloride. With certain substituted phenylhydrazones of ethyl pyruvate better results were obtained by adding the zinc chloride to a solution of the hydrazone in α-methylnaphthalene and heating the mixture in an oil-bath. In two cases, however, it was found expedient to reflux the hydrazone with absolute ethanol and concentrated sulphuric acid, in order to bring about ring closure and indole formation.

2-Methylindole

The phenylhydrazone of acetone was obtained in 86% yield (straw-coloured oil, b.p. 139° to 142° C. (18 mm.)) from the condensation of phenylhydrazine with acetone previously distilled over potassium permanganate. It (24 gm.) was mixed with freshly fused zinc chloride (27.3 gm.) and heated over a bare flame until the vigorous exothermic reaction started. The flask was withdrawn from the flame and shaken gently until the reaction had subsided and the reaction mixture solidified. The indole was separated from the mixture by steam distillation and extraction from the distillate with ether. It consisted of an oil that crystallized on cooling. Wt. 11.0 gm., yield 53%. 2-Methylindole was distilled *in vacuo*, b.p. 140° C. (1 mm.), and the crystalline distillate recrystallized from a mixture of absolute ether and petroleum ether, m.p. 62° C.* It yielded a pure picrate without further recrystallization.

3-Methylindole

Propylidenephenylhydrazone (27 gm., b.p. 135° to 145° C. (5 mm.)), prepared in 84% yield from phenylhydrazine and propionaldehyde, was fused with zinc chloride and the 3-methylindole isolated from the mixture by steam distillation. It was distilled, b.p. 100° to 110°C. (1 mm.), and twice crystallized from petroleum ether.

2,3-Dimethylindole

The phenylhydrazone of methyl ethyl ketone (23 gm., b.p. 146°C. (16 mm.)), prepared in 71% yield, was fused with zinc chloride (27.3 gm.) and the indole

All melting points are corrected.

obtained by steam-distillation of the mixture, wt. 17.6 gm., yield 85%. It was sublimed (132° to 133° C. (0.5 mm.)) and recrystallized from ether–petroleum-ether. This indole can also be prepared by the method of Tyson (15).

1,2- and 1,3-Dimethylindole

These two indoles were prepared by fusion with zinc chloride of the N-methylphenylhydrazones of acetone (b.p. 68° to 71° C. (3 mm.)) and of propionaldehyde (b.p. 101° to 105° C. (5 mm.)).

2,5- and 3,5-Dimethylindole

The necessary *p*-tolylhydrazine was prepared from purified *p*-toluidine by diazotization and reduction of the product with stannous chloride and hydrochloric acid (13). The *p*-tolylhydrazone of acetone was obtained in 82% yield, b.p. 125° to 135° C. (2 mm.), m.p. 45° to 46° C. Raschen (20) gives m.p. 50° to 52° C. The *p*-tolylhydrazone of propionaldehyde (yield 79%) boiled at 127° to 135° C. (2 mm.). Fusion with zinc chloride of the appropriate *p*-tolylhydrazones produced respectively 2,5-dimethylindole (yield 28%, sublimes at 90° to 103° C. (0.5 mm.) m.p. 115° C.) and 3,5-dimethylindole (yield 33%, b.p. 78° to 85° C. (1 mm.), long, colourless needles, m.p. 75° C.).

2,7- and 3,7-Dimethylindole

The required *o*-tolylhydrazine was prepared according to the directions of McPherson and Stratton (13) and condensed with acetone and with propionaldehyde to obtain the corresponding *o*-tolylhydrazones. Isopropylidene *o*-tolylhydrazone (72% yield, b.p. 128° to 133° C. (4 mm.), m.p. 49° C.) fused with zinc chloride gave rise to 2,7-dimethylindole, a light-yellow oil, b.p. 80° to 86° C. (0.5 mm.), yield 6.1%. The picrate separated as slender red needles, m.p. 156.5° C. Calc. for $C_{16}H_{14}O_7N_4$: C, 51.34; H, 3.74%. Found: C, 51.16, 51.33; H, 3.91, 3.92%.

Fropylidene *o*-tolylhydrazone (yield 97%, b.p. 122° to 132° C. (2 mm.)) when fused with zinc chloride produced 3,7-dimethylindole, b.p. 80° to 95° C. (0.5 mm.), in 25% yield.

Ethyl Pyruvate

Freshly distilled pyruvic acid (b.p. 64° to 67° C. (15 mm.), 616 gm.) was added to absolute ethanol (322 gm.) and refluxed for 16 hr. Ethyl pyruvate was isolated by fractionation of the product *in vacuo*, b.p. 51.5° to 53° C. (16.5 mm.), wt. 385 gm., yield 47%.

7-Methylindole

A mixture of *o*-tolylhydrazine (27 gm.) and ethyl pyruvate (29 gm.) was stirred while cooling under the tap. Ether and anhydrous sodium sulphate were added, and the solution filtered and diluted with petroleum ether. The crystalline *o*-tolylhydrazone that separated was filtered and recrystallized from 50% ethanol and from petroleum ether; colourless needles, melting at 76° to 77° C., wt. 38 gm., yield 78%. Calc. for $C_{12}H_{16}O_2N_2$: C, 65.45; H, 7.27; N, 12.73%. Found: C, 65.55, 65.25; H, 7.28, 7.40; N, 12.76%.

The ether–petroleum-ether mother-liquor, on concentration, yielded a further crop of crystals which, recrystallized from ether–petroleum-ether and from aqueous ethanol, was obtained as yellow needles melting at 62° C. In admixture with the hydrazone melting at 77° C., it melted at 50° to 55° C. Found: C, 65.83, 65.40; H, 7.47, 7.50; N, 12.86%. This second compound seems to be isomeric with, but different from, the higher melting hydrazone.

o-Tolylhydrazone of ethyl pyruvate (m.p. 77° C., in batches of about 10 gm.) was heated with fused zinc chloride at 220° C. in an oil-bath. The combined product of the reaction was warmed with dilute hydrochloric acid and, after cooling, extracted with ether. The combined ether extract was washed with dilute sodium hydroxide solution, with water, dried over anhydrous potassium carbonate, and distilled to dryness. The residual ethyl 7-methylindole-2-carboxylate was distilled *in vacuo* and the distillate which crystallized on cooling was recrystallized once from ether–petroleum-ether and twice from methanol, from which it separated as short, colourless needles melting at 131° C. Calc. for $C_{12}H_{13}O_2N$: C, 70.94; H, 6.40%. Found: C, 70.31, 70.28; H, 6.42, 6.69%. The sodium hydroxide washings from the ether solution, when acidified with hydrochloric acid, yielded a small quantity of 7-methylindole-2-carboxylic acid.

The ester, when saponified with alcoholic potassium hydroxide, yielded the corresponding acid which, twice recrystallized from water, consisted of small, colourless flat needles melting at 176° C. Calc. for $C_{10}H_9O_2N$: C, 68.57; H, 5.14%. Found: C, 68.43, 68.80; H, 5.28, 5.07%.

7-Methylindole-2-carboxylic acid (2 gm.) was decarboxylated by refluxing in the presence of quinoline (2 gm.) and a trace of copper-bronze for one hour in a metal-bath at 250° to 260° C. The mixture was cooled, dissolved in ether, and the filtered ether solution washed repeatedly with 5% hydrochloric acid, with 5% sodium carbonate solution, and with water. It was dried over anhydrous potassium carbonate, the ether distilled off and the residue distilled *in vacuo*. There was obtained 0.9 gm. of 7-methylindole (yield 60%) which, recrystallized from ether–petroleum-ether, formed glistening plates melting at 85° C. Calc. for C_9H_9N : C, 82.44; H, 6.87%. Found: C, 82.87, 82.81; H, 7.17, 7.14%. The picrate of 7-methylindole, recrystallized from methanol containing a little petroleum ether, consisted of red needles melting at 177.5° C.

1,7-Dimethylindole

o-Toluidine was converted to N-methyl-*o*-toluidine in 75% yield by the action of dimethyl sulphate. N-methyl-*o*-toluidine was purified by recrystallization of its acetyl derivative (m.p. 58° C.) and hydrolysis back to the base, b.p. 98.5° to 99° C. (17 mm.). Treatment of N-methyl-*o*-toluidine with sodium nitrite in hydrochloric acid (9) produced N-nitroso-methyl-*o*-toluidine (yield, 88%) which when reduced with zinc dust in acetic acid (8) gave rise to N-methyl-*o*-tolylhydrazine (yield, 35%). N-Methyl-*o*-tolylhydrazine (32

gm.) was condensed with ethyl pyruvate (30 gm.) and the product fractionated *in vacuo*. The N-methyl-*o*-tolylhydrazone produced, b.p. 140° to 141° C. (4 mm.), is a yellow oil; wt. 44 gm., yield, 80%. It was dissolved in α-methylnaphthalene and the solution heated with zinc chloride in an oil-bath at 150° C. for 30 min. The cooled mixture was washed repeatedly with ether and the combined ether solution distilled to dryness. The residual mixture of α-methylnaphthalene and crude ester was refluxed with alcoholic potassium hydroxide. To the cooled solution, water and ether were added and after thorough mixing the aqueous layer was separated. The ether solution was washed twice more with water and the combined aqueous solution acidified with hydrochloric acid. The precipitated 1,7-dimethylindole-2-carboxylic acid was extracted with ether and sublimed *in vacuo* (190° to 195° C. (1.5 mm.)). The sublimation was accompanied by some decomposition and the formation of a small quantity of 1,7-dimethylindole. The acid was recrystallized once from dilute acetic acid and twice from methanol from which it separated as short, colourless needles melting at 218.5° C. (decomp.), wt. 2.9 gm. Calc. for $C_{11}H_{11}O_2N$: C, 69.84; H, 5.82; N, 7.41%. Found: C, 70.31, 70.13; H, 6.01, 6.32; N, 7.33, 7.52%.

Decarboxylation of the acid by heating with quinoline and copper-bronze gave rise to 1,7-dimethylindole, b.p. 80° to 83° C. (1 mm.). Crystallized from ether–petroleum-ether it separated as colourless, well formed prisms melting at 78° C.; wt. 1.7 gm., yield, 76%. Calc. for $C_{10}H_{11}N$: C, 82.76; H, 7.59; N, 9.66%. Found: C, 82.61, 82.51; H, 7.76, 7.80; N, 9.86, 9.79%. It yielded a picrate which, recrystallized from methanol, melted at 154.5° C. Calc. for $C_{16}H_{14}O_7N_4$: C, 51.34; H, 3.74%. Found: C, 52.17; H, 4.13%.

4,7-Dimethylindole

p-Xylylhydrazine (10 gm.), prepared from *p*-xylidine by diazotization and subsequent reduction, was condensed with ethyl pyruvate (9 gm.) and the resulting xylylhydrazone (6) obtained as pale-yellow plates melting at 90° C., wt. 14 gm.; yield, 81%. Calc. for $C_{13}H_{18}O_2N_2$: C, 66.67; H, 7.69%. Found: C, 66.91, 67.16; H, 8.13, 8.07%.

An attempt to close the pyrrol ring by heating the xylylhydrazone dissolved in α-methylnaphthalene with zinc chloride resulted only in the partial saponification of the ester-xylylhydrazone to the xylylhydrazone of pyruvic acid. The latter was crystallized once from aqueous methanol and twice from petroleum ether from which it separated as small, colourless, prismatic needles melting at 173° C. Calc. for $C_{11}H_{14}O_2N_2$: N, 13.59%. Found: N, 13.72, 13.79%. Refluxing the acid xylylhydrazone (2.8 gm.) overnight with absolute ethanol and concentrated sulphuric acid not only caused esterification but also brought about ring closure and the formation of ethyl 4,7-dimethylindole-2-carboxylate, which sublimed at 105° to 110° C. (<1 mm.), and crystallized from ether–petroleum-ether as small, colourless plates melting at 142° C. Wt. 0.4 gm. Calc. for $C_{13}H_{15}O_2N$: C, 71.89; H, 6.91; N, 6.45%.

Found: C, 71.92, 71.80; H, 7.02, 6.97; N, 6.62, 6.65%. Refluxing the *p*-xylylhydrazone of ethyl pyruvate (5 gm.) with absolute ethanol (55 cc.) and concentrated sulphuric acid (5 cc.) for six hours yielded 1.08 gm. of ethyl 4,7-dimethylindole-2-carboxylate.

Ethyl 4,7-dimethylindole-2-carboxylate (1.3 gm.) on saponification yielded 4,7-dimethylindole-2-carboxylic acid (wt. 1.2 gm., yield 92.3%), which crystallized from aqueous ethanol as colourless, very small crystals, m.p. 208° C.

Decarboxylation of 4,7-dimethylindole-2-carboxylic acid by the usual method produced 4,7-dimethylindole in 94% yield. It crystallized from ether–petroleum-ether as very small, colourless crystals melting at 102° C. Plancher and Caravaggi (18) give the melting point as 101° to 102° C. but they did not prepare the picrate.

6,7-Dimethylindole

The 3-nitro-*o*-xylene necessary for this synthesis contained some 4-nitro-*o*-xylene, and a good separation was effected by a careful fractionation of the product through a Stedman column. The pure 3-nitro-*o*-xylene boiled at 72° C. (1 mm.) (347 gm. was obtained from 450 gm. of technical material).

3-Nitro-*o*-xylene was converted to 3-*o*-xylylhydrazine by a series of reactions parallel to those used for the synthesis of *p*-xylylhydrazine outlined above. 3-*o*-Xylylhydrazine (64 gm., m.p. 111° to 112° C.) was condensed with ethyl pyruvate (68 gm.) and the resulting 3-*o*-xylylhydrazone of ethyl pyruvate (77 gm., yield 70%) crystallized twice from petroleum ether and once from aqueous methanol, from which it separated as white needles with a slight yellowish cast, m.p. 75.5° C. Calc. for $C_{13}H_{18}O_2N_2$: N, 11.97%. Found: N, 11.98%. An attempt to effect ring closure by heating in α-methylnaphthalene solution with zinc chloride was unsuccessful and resulted in the partial hydrolysis of the ester-xylylhydrazone to the corresponding 3-*o*-xylylhydrazone of pyruvic acid, which was crystallized from acetic acid and from methanol from which it separated as fine yellow needles, m.p. 194° C. Calc. for $C_{11}H_{14}O_2N_2$: C, 64.08; H, 6.80%. Found: C, 63.92, 64.00; H, 6.95, 7.02%.

3-*o*-Xylylhydrazone of pyruvic acid (5 gm.) was refluxed for five hours with absolute ethanol (50 cc.) and concentrated sulphuric acid (5 cc.). The contents of the flask was poured into water and the gummy precipitate separated by ether extraction and distilled *in vacuo*. The ethyl 6,7-dimethylindole-2-carboxylate thus obtained (wt. 1.4 gm.), after crystallization once from ether–petroleum-ether and twice from methanol, consisted of colourless needles, m.p. 163° C. Calc. for $C_{13}H_{15}O_2N$: C, 71.89; H, 6.91; N, 6.45%. Found: C, 71.78, 71.89; H, 6.99, 7.00; N, 6.49, 6.50%. The use of larger quantities of 3-*o*-xylylhydrazone of pyruvic acid in the ring closure reaction reduced the yield considerably.

Saponification of ethyl 6,7-dimethylindole-2-carboxylate yielded the corresponding acid, which crystallized from aqueous methanol as colourless micro-

scopic crystals, m.p. 216° C. Calc. for $C_{11}H_{11}O_2N$: C, 69.84; H, 5.82%. Found: C, 70.63, 70.40; H, 6.10, 6.04%. On decarboxylation the foregoing acid yielded 6,7-dimethylindole (79% yield) which, crystallized from ether–petroleum-ether, consisted of colourless plates, m.p. 70° C. Calc. for $C_{10}H_{11}N$: C, 82.75; H, 7.59%. Found: C, 83.59, 83.66; H, 7.65, 7.54%. 6,7-Dimethylindole picrate crystallized from methanol as dull red needles, m.p. 164° C.

1-Methylindole

Condensation of N-methylphenylhydrazine (24.4 gm.) with ethyl pyruvate (23.2 gm.) gave rise to the N-methylhydrazone of ethyl pyruvate (37.5 gm., yield 85%). After distillation, b.p. 139° to 149° C. (3 mm.), it consists of an orange-coloured oil. Ring closure was effected by heating the N-methylphenylhydrazone of ethyl pyruvate in α-methylnaphthalene with zinc chloride. The product when saponified with alcoholic potassium hydroxide yielded 1-methylindole-2-carboxylic acid (5.8 gm.) which after sublimation (155° to 160° C. (<1 mm.)), and crystallization from ethanol, melted at 214° C. Calc. for $C_{10}H_9O_2N$: C, 68.57; H, 5.14%. Found: C, 68.28; H, 4.98%. Decarboxylation of the acid in quinoline in the presence of copper-bronze produced 1-methylindole, 4.23 gm., yield, 97%, which boiled at 70° to 75° C. (2 mm.). 1-Methylindole has also been prepared by a different method (15).

The Syntheses by the Tyson Method

The method of indole ring closure developed by Tyson (23, 24) consists in heating the appropriate formyltoluidide with a potassium alcoholate. It has been shown by Marion and Ashford (15) to be applicable to formyltoluidides methylated on the nitrogen for the production of 1-methylindoles, to acetyltoluidides for the formation of 2-methylindoles, and to formylanilides carrying an ethyl group in the 2-position for the production of 3-methylindoles. The reaction works equally well whether potassium ethylate or potassium *tert*-butylate is used.

4-Methylindole

3-Nitro-*o*-xylene was reduced with iron powder and acetic acid (4) to the corresponding xylidine. 3-*o*-Xylidine (30 gm.) was added to 97% formic acid (12 cc.) and the mixture heated on the steam-bath overnight. After cooling, the product was dissolved in benzene and the solution washed with water, dried over anhydrous sodium sulphate, and concentrated to a small volume. Addition of petroleum ether to incipient turbidity caused the crystallization of 3-formamino-*o*-xylene which, after recrystallization from a mixture of benzene and petroleum ether, consisted of short colourless needles, m.p. 105° C., wt. 25.2 gm. Calc. for $C_9H_{11}ON$: C, 72.47; H, 7.38; N, 9.39%. Found: C, 72.65, 72.49; H, 7.42, 7.23; N, 9.34, 9.38%.

The formyl derivative (25.2 gm.) when heated with potassium (9.8 gm.) dissolved in absolute ethanol (110 cc.) according to the directions of Tyson (24) was converted to 4-methylindole, a yellowish oil, b.p. 90° C. (<1 mm.), wt. 3.75 gm. Even at −60° C., 4-methylindole did not crystallize. The

picrate separated from methanol–ether as bright red needles, m.p. 194° C. Calc. for $C_{15}H_{12}O_7N_4$: N, 15.55%. Found: N, 15.51%.

2,4-Dimethylindole

Treatment of 3-*o*-xylidine (12 gm.) with acetic anhydride in benzene solution produced 3-acetamino-*o*-xylene (13 gm., yield 80%), which crystallized from benzene–petroleum-ether as colourless needles, m.p. 135.5° C. Yokoyama (25) reports a melting point of 133° C. 3-Acetamino-*o*-xylene, by heating with potassium *tert*-butylate, was converted to 2,4-dimethylindole, a pale-yellow oil, b.p. 94° to 96° C. (<1 mm.) (yield 56%). The picrate crystallized from ether–petroleum-ether as bronze-red needles, m.p. 164.5° C. Calc. for $C_{16}H_{14}O_7N_4$: C, 51.34; H, 3.74%. Found: C, 51.25, 51.54; H, 3.90, 3.89%.

1,4-Dimethylindole

3-Formylamino-*o*-xylene (36 gm.) was refluxed with alcoholic potassium hydroxide and methyl iodide as described by Bamberger and Wulz (2), and the 3-formylmethylamino-*o*-xylene (18 gm., yield 46%) produced was distilled *in vacuo*. It consists of a colourless oil, b.p. 97° to 99° C. (2 mm.). Calc. for $C_{10}H_{13}ON$: C, 73.62; H, 7.98; N, 8.59%. Found: C, 73.47, 73.62; H, 8.10, 8.06; N, 8.69, 8.69%. 3-Formylmethylamino-*o*-xylene when heated with potassium ethylate gave rise to 1,4-dimethylindole, a pale-yellow oil, b.p. 73° to 75° C. (0.2 mm.). The picrate separated from ether–petroleum-ether as orange-coloured needles, m.p. 187° C. Calc. for $C_{16}H_{14}O_7N_4$: C, 51.34; H, 3.74%. Found: C, 50.87, 51.04; H, 3.64, 3.67%.

6-Methylindole

p-Xylidine (2,5-dimethylaniline) was purified by the methods of Hodgkinson and Limpach (10) and of Morgan and Hickinbottom (17) and then fractionated *in vacuo*. The fraction boiling at 73° to 74° C. (2 mm.) was used. *p*-Xylidine by treatment with 87% formic acid, as already described, was converted to formamino-*p*-xylene, which crystallized from benzene–petroleum-ether as soft colourless needles, m.p. 119° C. Calc. for $C_9H_{11}ON$: N, 9.39%. Found: N, 9.35%.

Heating formamino-*p*-xylene with potassium *tert*-butylate produced 6-methylindole in 36% yield. The indole is a pale-yellow oil, b.p. 75° to 78° C. (1 mm.), which yields a picrate crystallizing from ether–petroleum-ether as orange-coloured needles, m.p. 161.5° C. Calc. for $C_{15}H_{12}O_7N_4$: C, 50.00; H, 3.33%. Found: C, 50.00, 50.17; H, 3.13, 3.43%.

2,6-Dimethylindole

Acetamino-*p*-xylene was prepared in 83% yield from *p*-xylidine (2,5-dimethylaniline) and acetic anhydride. It separates from aqueous methanol as long, colourless needles, m.p. 142° C. It (22.5 gm.) was converted, by heating with potassium *tert*-butylate, to 2,6-dimethylindole (10 gm., yield 50%), which crystallizes from ether–petroleum-ether as long, colourless needles, m.p. 88.5° C. Calc. for $C_{10}H_{11}N$: N, 9.65%. Found: N, 9.52.

9.67%. The picrate is obtained by crystallization from ether–petroleum-ether as brick-red needles, m.p. 138.5° C. Calc. for $C_{16}H_{14}O_7N_4$: C, 51.34; H, 3.74%. Found: C, 51.39, 51.49; H, 3.88, 3.76%.

1-6-Dimethylindole

Formylmethylamino-*p*-xylene, prepared by refluxing formylamino-*p*-xylene with alcoholic potassium hydroxide and methyl iodide (2) is a colourless oil, b.p. 99° to 100.5° C. (3 mm.). Yield, 51%. Calc. for $C_{10}H_{13}ON$: C, 73.62; H, 7.98; N, 8.59%. Found: C, 73.82, 73.61; H, 8.12, 7.90; N, 8.77, 8.78%. With the aid of potassium *tert*-butylate it was converted to 1,6-dimethylindole, which is a yellow oil, b.p. 68° to 73° C. (0.2 mm.). The picrate crystallizes from absolute ether as dark red needles, m.p. 148° C. Calc. for $C_{16}H_{14}O_7N_4$: N, 14.97%. Found: N, 14.91%.

5-Methylindole

2,4-Dimethylaniline was prepared by the nitration of *m*-xylene and reduction of the 2,4-dimethylnitrobenzene with tin and hydrochloric acid. The resulting 2,4-dimethylaniline was purified through its acetate (14, 15) and distilled, b.p. 76.5° to 78° C. (3 mm.). It was converted by the usual method to 2,4-dimethylformanilide, which crystallizes from benzene as large colourless prisms, m.p. 115° to 116° C. Calc. for $C_9H_{11}ON$: N, 9.39%. Found: N, 9.38, 9.47%. 2,4-Dimethylformanilide, with the aid of potassium alcoholate, gives rise to 5-methylindole (yield 27%), which crystallizes from ether–petroleum-ether as glistening prismatic needles, m.p. 59.5° C. The picrate was obtained as red needles, m.p. 161.5°C. Calc. for $C_{15}H_{12}O_7N_4$: N, 15.55%. Found: 15.53, 15.58%.

1,5-Dimethylindole

4-Formylmethylamino-*m*-xylene was obtained by refluxing a mixture of 2,4-dimethylformanilide with alcoholic potassium hydroxide and methyl iodide (2). It is a colourless oil with a slight yellowish cast, b.p. 99° to 100° C. (2 mm.). Yield, 29%. Calc. for $C_{10}H_{13}ON$: C, 73.62; H, 7.98; N, 8.59%. Found: C, 73.55, 73.61; H, 8.07, 8.09; N, 8.72, 8.79%. Heating 4-formylmethylamino-*m*-xylene (21.5 gm.) with potassium ethylate produced a very low yield of 1,5-dimethylindole (0.32 gm.), which is a light yellow oil, b.p. 60° to 65° C. (1 mm.). The picrate separated from ether–petroleum-ether as red needles, m.p. 151° C. Calc. for $C_{16}H_{14}O_7N_4$: C, 51.34; H, 3.74%. Found: C, 51.12; H, 3.95%.

2,3,5-Trimethylaniline

The pseudocumidine (2,4,5-trimethylaniline) necessary as a starting material was obtained by the careful fractionation of commercial 'cumidine' through a Stedman column. The fraction boiling at 106° to 110° C. (11 mm.) crystallized when cooled in dry-ice and acetone. The crystals were filtered through a cooled fritted glass funnel and the filtrate was refractionated. A further fraction, b.p. 106° to 110° C. (11 mm.), thus obtained yielded a second crop of crystals of 2,4,5-trimethylaniline. The combined crystalline material,

recrystallized from petroleum ether, melted at 66° C. It was converted (yield, 84%) to 2,4,5-trimethylacetanilide, which crystallized from dilute acetic acid as fine, colourless needles, m.p. 164° C. (11) and this, when nitrated at 0° C. with concentrated nitric acid produced 6-nitro-2,4,5-trimethylacetanilide (12), which, after crystallization from ethanol, melted at 199° C., yield 82%. Hydrolysis of this nitro product with concentrated sulphuric acid (11) yielded 6-nitro-2,4,5-trimethylaniline (yield 63%) as bright orange-coloured crystals, m.p. 48° C. The amino group was removed by Mai's method (14) applied according to the directions of Adams and Kornblum (1). 6-Nitro-2,4,5-trimethylaniline (30.3 gm.) was heated on the steam-bath with 1 : 1 hydrochloric acid (500 cc.) and, when most had dissolved, the mixture was cooled with vigorous mechanical stirring. The mixture was diazotized at 5° to 10° C. with a solution of sodium nitrite (12.5 gm.) in water (30 cc.) added slowly in the course of 20 min. The stirring was continued for 30 min. more and the solution rapidly filtered and added to 30% hypophosphorous acid (325 cc.). The resulting solution was kept in the refrigerator for 10 hr. and then at room temperature for 11 hr. Cracked ice was added, the solution basified with sodium hydroxide, and the oil that separated was collected in ether. The ether extract was washed with water, dried over anhydrous potassium carbonate, and distilled to dryness. The residual nitropseudocumene (6-nitro-1,2,4-trimethylbenzene) was distilled *in vacuo*, b.p. 139° to 140° C. (7 mm.), wt. 17 gm., yield 61%. Reduction of the nitropseudocumene with iron powder and glacial acetic acid gave a 92% yield of the corresponding pseudocumidine (2,3,5-trimethylaniline), m.p. 39° C.

4,6-Dimethylindole

2,3,5-Trimethylaniline (15 gm.) was heated with 87% formic acid (6 cc.) and the resulting 2,3,5-trimethylformanilide (17.2 gm., yield 95%) crystallized from benzene, m.p. 135.5° C. Calc. for $C_{10}H_{13}ON$: N, 8.59%. Found: N, 8.65%. Heating 2,3,5-trimethylformanilide (17 gm.) with potassium (6 gm.) and *tert*-butyl alcohol (125 cc.) produced 4,6-dimethylindole, a yellowish oil, b.p. 90° to 95° C. (0.4 mm.), wt. 1.95 gm. The picrate separated from methanol in long dull dark-red needles, m.p. 169° C. Calc. for $C_{16}H_{14}O_7N_4$: C, 51.34; H, 3.74%. Found: C, 51.59, 51.45; H, 3.95, 4.06%.

*5,6-Dimethylindole**

Pseudocumidine melting at 66° C. (2,4,5-trimethylaniline) was converted by heating overnight with formic acid to 2,4,5-trimethylformanilide, which, after recrystallization from ethanol–ether, melted at 123.5° C. Yield, 90%. Heating 2,4,5-trimethylformanilide with potassium *tert*-butylate produced 5,6-dimethylindole, yield 17%. The indole distilled at 85° C. (0.5 mm.) and crystallized from petroleum ether as colourless needles, m.p. 64° C. The picrate crystallized as bright-red needles, m.p. 154.5° C. Calc. for $C_{16}H_{14}O_7N_4$: C, 51.34; H, 3.74%. Found: C, 52.04, 52.19; H, 4.20, 4.42%.

* *This indole was prepared by Dr. W. R. Ashford.*

5,7-Dimethylindole

Mesitylene and nitromesitylene were prepared according to the methods outlined in Organic Syntheses (16, 19). Nitromesitylene was reduced with tin and hydrochloric acid to mesidine (yield 58%) and the latter when heated with formic acid produced a 93% yield of formomesidide, which crystallized from ethanol as glistening, colourless needles, m.p. 182° C. Formomesidide (27 gm.) heated with potassium *tert*-butylate produced 5,7-dimethylindole (5 gm., yield 18%), which is a light-yellow oil, b.p. 80° to 84° C. (< 1 mm.). The picrate crystallized from ether–petroleum-ether in dark red needles, m.p. 183° C. Calc. for $C_{16}H_{14}O_7N_4$: C, 51.34; H, 3.74%. Found: C, 51.41, 51.14; H, 3.91, 3.82%.

Acknowledgments

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SULPHANILAMIDOPYRIMIDINES[1]

BY LEONARD C. LEITCH[2], BRUCE E. BAKER[3], AND LEO BRICKMAN[4]

Abstract

The syntheses of 2-sulphanilamido-4-hydroxypyrimidine, 2-sulphanilamido-4,6-dihydroxypyrimidine, and several other sulphanilamidopyrimidines are described. An improved method for the preparation of sulphaguanidine is given.

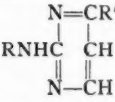
Introduction

In the course of a program of work relating to sulphanilamide derivatives, we found it necessary to synthesize 2-sulphanilamido-4-hydroxypyrimidine and 2-sulphanilamido-4,6-dihydroxypyrimidine. We found that acetylsulphanilyl chloride would not react either with 2-amino-4-hydroxypyrimidine, in agreement with the observation of Raiziss and Freifelder (10), or with 2-amino-4,6-dihydroxypyrimidine. 2-Sulphanilamido-4-hydroxypyrimidine (VI) was synthesized by the three methods outlined in Chart I.

In Scheme *A*, 2-amino-4-hydroxypyrimidine (I) was converted successively to 2-amino-4-chloropyrimidine (II) by means of phosphorus oxychloride, then to 2-amino-4-methoxypyrimidine (III) by reaction with sodium methoxide, and then to 2-acetylsulphanilamido-4-methoxypyrimidine (IV) by coupling with acetylsulphanilyl chloride. Compound (IV) was deacetylated to (V) and then demethylated to the desired 2-sulphanilamido-4-hydroxypyrimidine (VI).

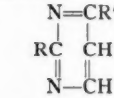
Chart I

Scheme A



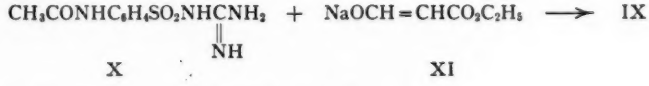
I R = H, R′ = OH
II R = H, R′ = Cl
III R = H, R′ = CH_3O–
IV R = $CH_3CONHC_6H_4SO_2$–, R′ = CH_3O–
V R = $NH_2C_6H_4SO_2$–, R′ = CH_3O–
VI R = $NH_2C_6H_4SO_2$–, R′ = OH

Scheme B



VII R = CH_3S–, R′ = OH
VIII R = $NH_2SO_2C_6H_4NH$–, R′ = OH
IX R = $CH_3CONHC_6H_4SO_2NH$–, R′ = OH

Scheme C



X XI

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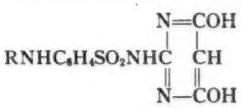
In Scheme *B*, 2-methylmercapto-4-hydroxypyrimidine (VII) was condensed with acetylsulphanilamide to give 2-acetylsulphanilamido-4-hydroxypyrimidine (IX), which was then deacetylated to (VI). If sulphanilamide was used in the condensation in place of acetylsulphanilamide, the product obtained was (VIII), substitution taking place on the amino rather than the amido group.

In Scheme *C*, acetylsulphaguanidine (X) was condensed with sodium formylacetic ester (XI) to give (IX), which was then deacetylated to the desired compound (VI). Ganapathi and co-workers (4) prepared this compound directly from sulphaguanidine and formylacetic ester.

The same three methods were applied in an attempt to synthesize 2-sulphanilamido-4,6-dihydroxypyrimidine but the results were not so gratifying. 2-Amino-4,6-dihydroxypyrimidine was substituted for (I) in Scheme *A* and was successfully carried through the first four steps of the reaction to 2-sulphanilamido-4,6-dimethoxypyrimidine, but attempts to demethylate this compound gave rise to decomposition products and none of the desired compound could be isolated.

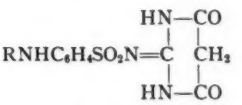
When 2-methylmercapto-4,6-dihydroxypyrimidine was substituted for (VII) in Scheme *B*, the desired reaction did not take place and only unchanged acetylsulphanilamide was recovered from the reaction mixture.

Applying Scheme *C*, it was found that acetylsulphaguanidine (X) reacted smoothly with malonic ester in the presence of sodium ethoxide to give 2-acetylsulphanilamido-4,6-dihydroxypyrimidine (XII).



XII R = CH_3CO–

XIII R = H



XIV R = CH_3CO–

XV R = H

On deacetylation, 2-sulphanilamido-4,6-dihydroxypyrimidine (XIII) was obtained. This same compound could be obtained directly in excellent yields by condensing sulphaguanidine with malonic ester in the presence of sodium ethoxide. Curiously enough, (XII) and (XIII) were soluble not only in alkalies and alkali carbonates, but in alkali bicarbonates as well. Furthermore, (XII) reacted with one equivalent, and (XIII) with two equivalents, of nitrous acid. This is in agreement with the observation of Traube (13), who found that 2-amino-4,6-dihydroxypyrimidine reacted with nitrous acid to form the 5-oximino derivative. Compounds (XII) and (XIII) probably exist mainly in the oxo forms (XIV) and (XV). The active methylene group situated between the two carbonyl groups in the pyrimidine ring would account for the reactions described above.

Pharmacology

2-Sulphanilamido-4-hydroxypyrimidine and 2-sulphanilamido-4,6-dihydroxypyrimidine, as well as sulphanilylthiourea (7), were tested *in vivo* against influenza and rabic virus infections in mice. No significant cures were found. Against *Pneumococcus* (McGovern type I) in mice, these compounds were inferior to sulphadiazine, and against *Streptococcus hemolyticus* they were inferior to sulphanilamide. The two pyrimidine compounds were ineffective when administered orally to malaria-infected ducks while the thiourea showed no activity when tested against *Trypanosoma equiperdum* in rats.

Experimental

Synthesis of 2-Sulphanilamido-4-hydroxypyrimidine

Scheme A

2-Amino-4-hydroxypyrimidine was prepared according to the directions of Roblin *et al.* (11). This was converted to 2-amino-4-chloropyrimidine as described by Gabriel and Colman (3), and then to 2-amino-4-methoxypyrimidine according to Adams and Whitmore (1). This compound was coupled with acetylsulphanilyl chloride and deacetylated to 2-sulphanilamido-4-methoxypyrimidine as described by Roblin and co-workers (12). The demethylation was carried out by dissolving the material in concentrated hydrochloric acid and evaporating the solution to dryness on the steam-bath (6). The residue was taken up in water and neutralized with sodium bicarbonate solution. The product, 2-sulphanilamido-4-hydroxypyrimidine, melted at 269° to 271° C.* either alone or admixed with the material prepared by means of Schemes *B* and *C*.

Scheme B

2-Methylmercapto-4-hydroxypyrimidine was prepared by the method of Wheeler and Merriam (15). This product (3.5 gm., 0.025 mole) was added in small portions to molten acetylsulphanilamide (5.5 gm., 0.025 mole) over a period of one hour, with good stirring. The mixture was kept immersed in an oil-bath at 230° C. Evolution of methylmercaptan took place slowly. The reaction mixture was poured into water and then digested with 2% aqueous sodium hydroxide (50 ml.) until solution was complete. The solution was filtered with carbon black and neutralized with dilute acetic acid. The crude 2-acetylsulphanilamido-4-hydroxypyrimidine weighed 4 gm. A sample recrystallized from water melted at 263° to 264° C. either alone or when mixed with the 2-acetylsulphanilamido-4-hydroxypyrimidine prepared under Scheme *C*.

Deacetylation with aqueous sodium hydroxide gave 2-sulphanilamido-4-hydroxypyrimidine melting at 270° to 272° C. It was identical with the material obtained by the other two methods.

* *Melting points are uncorrected.*

When the reaction was carried out by adding the 2-methylmercapto-4-hydroxypyrimidine to sulphanilamide in molten phenol at 175° C. (5), the product obtained was the isomeric N^4-(4-hydroxypyrimidine-2)-sulphanilamide. This product melted at 288° to 290°C. It did not give an azo colour reaction with nitrous acid and 2-naphthol and could not be titrated with standard sodium nitrite solution; this indicated the absence of a free amino group. Calc. for $C_{10}H_{10}O_3N_4S$: N, 21.0%. Found: N, 20.7%.

Scheme C

Sodium formylacetic ester was prepared by adding dropwise a mixture of ethyl acetate (44 gm., 0.5 mole) and ethyl formate (37.0 gm., 0.5 mole) to finely divided sodium (11.5 gm., 0.5 mole) in absolute ether (100 ml.). The reaction mixture was cooled by means of running water. When the sodium had completely reacted, the ether was distilled off and the residue dissolved in absolute ethanol. Acetylsulphaguanidine (40 gm., 0.15 mole) was added and the mixture stirred and refluxed for five hours. The suspended solids were filtered off, washed with a little ethanol, stirred up with warm water (500 ml.) and filtered. The insoluble material was unchanged acetylsulphaguanidine and weighed 13.0 gm. The filtrate on acidification gave 19.5 gm. (40.5%) of 2-acetylsulphanilamido-4-hydroxypyrimidine. A sample recrystallized from water melted at 263° to 264°C. Calc. for $C_{12}H_{12}O_4N_4S$: N, 18.2%. Found: N, 18.2%.

The acetyl compound (12 gm., 0.039 mole) was refluxed for 30 min. with 50 ml. of 4 *N* hydrochloric acid. The solution was filtered with carbon black and neutralized with a solution of sodium acetate. The yield of 2-sulphanilamido-4-hydroxypyrimidine was 9.0 gm. (86.5%). A sample recrystallized from water melted at 269° to 270°C., in good agreement with the melting point given by Ganapathi (4) for this compound. Calc. for $C_{10}H_{10}O_3N_4S$: total N, 21.0%; amino N, 5.26%. Found: total N, 20.9%; amino N, 5.4%.

Synthesis of 2-Sulphanilamido-4,6-dihydroxypyrimidine

Scheme A

2-Amino-4,6-dihydroxypyrimidine was prepared as described by Michael (9). This was converted to 2-amino-4,6-dichloropyrimidine according to the directions of Büttner (2) and then to 2-amino-4,6-dimethoxypyrimidine (1, 6). This was coupled with acetylsulphanilyl chloride (12) to give 2-acetylsulphanilamido-4,6-dimethoxypyrimidine, which melted at 235° to 236° C. after one recrystallization from ethanol. Calc. for $C_{14}H_{16}O_5N_4S$: N, 15.9%. Found: N, 15.7%. Deacetylation with aqueous sodium hydroxide gave 2-sulphanilamido-4,6-dimethoxypyrimidine, which after recrystallization from ethanol melted at 170° to 172° C. Calc. for $C_{12}H_{13}O_4N_4S$: N, 18.1%. Found: N, 17.9%. All attempts to demethylate this product resulted in decomposition.

Scheme B

Thiobarbituric acid was prepared by the method of Michael (8). The product was soluble in bicarbonate as well as carbonate. It was converted

to 2-methylmercapto-4,6-dihydroxypyrimidine according to the directions of Wheeler and Jamieson (14). It was treated with molten acetylsulphanilamide as described under Scheme *B* above. The only product which could be isolated from the reaction mixture was unchanged acetylsulphanilamide.

Scheme C

To a solution of sodium ethoxide (from 40 gm. of sodium and 600 ml. of ethanol) was added malonic ester (80 gm., 0.5 mole) followed by acetylsulphaguanidine (90 gm., 0.35 mole). The mixture was stirred and refluxed for five hours. After cooling, the solution was filtered, the insoluble product taken up in water (600 ml.) and carefully acidified with 6 *N* hydrochloric acid. There was obtained 73 gm. (64%) of product. The 2-acetylsulphanilamido-4,6-dihydroxypyrimidine thus obtained had no definite melting point, but decomposed gradually above 162° C. It was soluble in alkalies, alkali carbonates, and bicarbonates, but insoluble in acids. It was purified by reprecipitation from sodium hydroxide solution. Calc. for $C_{12}H_{12}O_5N_4S$: N, 17.2%. Found: N, 17.3%. The product, dissolved in acetic acid, was titrated with sodium nitrite solution. One mole of the material consumed 0.96 mole of sodium nitrite.

The product (12.5 gm., 0.0385 mole) was deacetylated by heating to 95° C. with sodium hydroxide (6 gm., 0.15 mole) in water (25 ml.) for several minutes and then allowing the solution to cool slowly. After diluting and filtering with carbon black, it was acidified with dilute hydrochloric acid. The yield of 2-sulphanilamido-4,6-dihydroxypyrimidine was 10 gm. (91.7%); it melted at 248° to 249° C. Calc. for $C_{10}H_{10}O_4N_4S$: N, 19.9%. Found: N, 19.5%. On titration with standard sodium nitrite, one mole of the material consumed 2.06 equivalents of sodium nitrite.

When the reaction with malonic ester was carried out as described above but substituting sulphaguanidine for acetylsulphaguanidine, there was obtained 88.5% of 2-sulphanilamido-4,6-dihydroxypyrimidine melting at 250° to 251°C. It was identical in every respect with the material obtained by deacetylation as described above.

The sulphaguanidine used in the above experiment was prepared by the following modification of the method previously described (7). In this new method, sulphaguanidine was obtained directly in one operation from calcium acetylsulphanilylcyanamide and ammonia. The calcium acetylsulphanilylcyanamide (260 gm., 0.5 mole), prepared as described in the previous paper (7), was placed in a cast-iron steam-jacketted autoclave fitted with a high-speed agitator (600 r.p.m.) and a thermometer well. Concentrated ammonia water (200 ml.), diluted to 600 ml., was added and the mixture heated and stirred at 155° C. for four hours. At the end of this time, the reaction vessel was cooled to room temperature and the insoluble product was filtered from the reaction mixture. The crude sulphaguanidine, after drying at 110° C., weighed 105 gm. It was recrystallized from 2000 ml. of boiling water, using a small amount of carbon black to decolorize the solution. The pure sulphaguanidine, (90 gm., 42%), melted at 187° to 189° C.

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THE SYNTHESIS OF DI- AND TRITHIOLS[1]

By S. D. Simpson[2]

Abstract

In the course of a study of antidotes to lewisite the following thiols have been prepared from the corresponding di- and trihalides by the action of sodium hydrosulphide or ammonium hydrosulphide in ethanol under pressure: 1,2-dimercaptoethane, 1,2-dimercaptopropane, 1,3-dimercaptopropane, 1,2,3-trimercaptopropane, 1,2-dimercapto-*n*-butane, 2,2′-dimercaptoethyl ether, 3,3′-dimercaptopropyl ether, 2,2′-dimercaptoisopropyl ether, and 1,3-dimercapto-2-propanol.

1,2-Dimercaptopropane has been prepared by a new method, namely, by the interaction of ammonium hydrosulphide and allyl bromide. This method involves the addition of hydrogen sulphide to an unsaturated compound by the action of ammonium hydrosulphide in ethanol under pressure.

The toxicity of the above thiols and their antidotal activity to lewisite have been compared with those of 2,3-dimercaptopropanol (BAL, British anti-lewisite) and the results of these studies will be published later.

Introduction

Previous to World War II there was evidence that the toxicity of trivalent arsenical compounds is related to their reaction with essential thiol groups in the organism (12, 18). It had been shown that monothiols give some protection against the toxic action of arsenical compounds (3, 13, 19). In 1940, Peters, Stocken, and Thompson showed that 2,3-dimercaptopropanol is effective against the toxic actions of lewisite and other arsenical compounds. This dithiol subsequently became known as BAL (British anti-lewisite). A review of work on BAL has been published by Peters, Stocken, and Thompson (10). Waters and Stock (20) have described researches on BAL in the United States, and Canadian researches on BAL have been reviewed by Young (22). The thiols described herein were prepared in an attempt to find an agent that would be more suitable than 2,3-dimercaptopropanol as an antidote to lewisite, and in the hope that information would be gained concerning the relation of the structure of thiols to their toxicity and antidotal activity (14).

The synthesis of the following thiols is described below: 1,2-dimercaptoethane, 1,2-dimercaptopropane, 1,3-dimercaptopropane, 1,2,3-trimercaptopropane, 1,2-dimercapto-*n*-butane, 2,2′-dimercaptoethyl ether, 3,3′-dimercaptopropyl ether, 2,2′-dimercaptoisopropyl ether, and 1,3-dimercapto-2-propanol. The results obtained when the above-mentioned thiols and also 2,3-dimercaptopropanol were tested for their toxicity and antidotal actions have been reported (15) and will be published later. No reference has been found to the previous preparation of 1,2-dimercapto-*n*-butane, 2,2′-dimercaptoethyl ether, 3,3′-dimercaptopropyl ether, or 2,2′-dimercaptoisopropyl ether. All the thiols

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described herein were prepared by the interaction of organic halides and sodium hydrosulphide or ammonium hydrosulphide in ethanol under pressure.

Some of the thiols described herein have been prepared previously by the action of sodium or potassium hydrosulphide on the corresponding halide. These preparations, however, were carried out at atmospheric pressure in an open vessel (1, 2, 4-8, 11, 16, 21). Previous to the present work, investigators at Oxford had prepared several dithiols by the interaction of sodium hydrosulphide or ammonium hydrosulphide and the appropriate halide under pressure (10).

One dithiol, 1,2-dimercaptopropane, was prepared by the action of ammonium hydrosulphide on allyl bromide.

$$CH_2 : CH \,.\, CH_2Br + 2NH_4SH \longrightarrow CH_3 \,.\, CH(SH) \,.\, CH_2SH + NH_3 + NH_4Br.$$

The identity of the compound obtained in this reaction was established by analysis and by comparison of its properties with those of 1,2-dimercaptopropane and 1,3-dimercaptopropane prepared from the corresponding dihalogen compounds. The above type of reaction is of interest as it would appear to be applicable to the synthesis of other thiols.

Reagents and Methods

The thiols were synthesized by the methods described below. An organic halide was allowed to react with either sodium hydrosulphide or ammonium hydrosulphide in ethanol. Two molecules of ammonium or sodium hydrosulphide were used for each halogen atom in the reaction. The reaction was carried out by heating the reagents in a sealed vessel. At the end of the reaction the thiol was separated from the alcoholic solution by (i) the evaporation of the alcohol under reduced pressure and extraction of the thiol with ether, or (ii) addition of aqueous sodium hydroxide solution, evaporation to dryness under reduced pressure, acidification of the residue, and extraction of the thiol with ether, or (iii) addition of three volumes of water to the alcoholic solution, removal of the thiol that separated, followed by extraction of the remaining thiol with ether. The ether solution of the thiol was dried over anhydrous sodium sulphate, filtered, and then evaporated under reduced pressure. Each thiol was then redistilled until its purity, determined by iodine titration, reached a maximum. It was sometimes found that a thiol was difficult to purify by distillation. In such a case it was dissolved in ethanol, aqueous sodium hydroxide solution was added, the solution was evaporated to dryness under reduced pressure, and the thiol was liberated from the residue by acidification; it was then extracted with ether and purified by the method described above.

Preparation of Ammonium Hydrosulphide

This reagent was prepared by the method of Thomas and Riding (17). A stock solution of ammoniacal alcohol was obtained by bubbling ammonia gas (dried by passage over quicklime) through ethanol until a concentration of

50 to 60 gm. of ammonia per litre of solution was obtained. The concentration of ammonia in the solution was determined by titrating aliquot portions of the solution with hydrochloric acid, using methyl orange as indicator. Hydrogen sulphide, dried by passage over anhydrous calcium chloride, was passed through small portions of the ammoniacal ethanol at 0° C. Glistening white crystals formed which rapidly turned yellow, probably owing to the formation of polysulphides. The crystals were separated by filtration. Both the filtrate and a solution of the crystals were analysed by the titration of aliquot portions with hydrochloric acid and with iodine solution. It was found that analyses of the filtrate from the suspension of ammonium hydrosulphide in ethanol showed that the value of the ratio $NH_3 : H_2S$ was about 1.8, which corresponded to a solution containing $NH_4SH : 4(NH_4)_2S$. For a solution of the crystals the value of the ratio $NH_3 : H_2S$ was about 1.2, which corresponded to a solution containing $4NH_4SH : (NH_4)_2S$. The crystals were therefore used in the preparation of the thiols. They were added to ethanol at 0° C. in a glass pressure flask of 200 ml. capacity so that the volume of the suspension was 100 ml. The suspension was analysed for its ammonium hydrosulphide content and the halide was added in an amount such that there were two molecules of hydrosulphide for each halogen atom present. The flask was closed with a rubber stopper, which was then wired in position.

Preparation of Sodium Hydrosulphide

This reagent was prepared by the action of hydrogen sulphide on sodium hydroxide or sodium ethoxide in ethanol. After the theoretical amount of hydrogen sulphide had been absorbed, the solution was cooled to 0° C. and an excess of hydrogen sulphide was passed through. The advantage of using sodium ethoxide lay in the fact that an anhydrous solution of sodium hydrosulphide was obtained. Most of the thiol preparations in which sodium hydrosulphide was used were carried out in bombs of 650 and 1600 ml. capacity. Each bomb was provided with a gas valve for releasing the pressure previous to removing the contents of the bomb.

Determination of the Thiol Content of Preparations

The thiol content of the preparations was determined by titration with iodine solution in the following manner. A sample of about 0.05 gm. of the thiol was weighed accurately into a small glass cup, which was then dropped into 95% ethanol in a conical flask. The contents of the flask was shaken until all the thiol was dissolved in the ethanol. Iodine solution (0.1 *N*) was then run in slowly until a faint yellow colour remained. By means of this method it was possible to obtain duplicate values that did not differ by more than 0.5%. The iodine solution was standardized against sodium thiosulphate solution immediately before use.

Determination of Sulphur

A micro-Carius method was used to determine the sulphur content of the thiols. The procedure was based on that described by Niederl, Baum, McCoy, and Kuck (9).

Determination of Specific Gravity

The specific gravity of each thiol was determined in duplicate at 20° C., using pycnometers of approximately 0.6 ml. capacity, blown from tubing of 3 mm. inside diameter.

Experimental

In order to illustrate the methods used in the present work on the synthesis of thiols, the preparation of 1,2-dimercaptoethane from sodium hydrosulphide and that of 1,2-dimercaptopropane from ammonium hydrosulphide are described in detail.

1,2-Dimercaptoethane

Sodium hydroxide (40.0 gm.) was dissolved in ethanol (400 ml.) and converted to sodium hydrosulphide by the method described above. 1,2-Dibromoethane (47.0 gm.) was added and the solution was sealed in a bomb of 650 ml. capacity and heated at 30° C. for seven days and at 60° C. for four hours. The ethanol solution was filtered, three volumes of water were added to the filtrate, and the oil that separated was removed. The filtrate was made acid to Congo red with concentrated hydrochloric acid and was extracted with ether. The oil was combined with the ether extract and dried over anhydrous sodium sulphate. The ether was evaporated and the product was distilled under reduced pressure, b.p. 46° to 47° C. (16 mm.), 43° to 44° C. (14 mm.), sp.gr.$^{20}_{20}$ 1.124, yield 17%, purity 98.5% by iodine titration. Calc. for $C_2H_6S_2$: C, 25.49; H, 6.42; S, 68.10%. Found: C, 25.42; H, 6.58; S. 68.42%.

1,2-Dimercaptopropane

Preparation I. 3-Bromopropene (8.89 gm.) was added to 100 ml. of a suspension of ammonium hydrosulphide (15.0 gm.) in ethanol in a 200 ml. glass pressure flask. The flask was sealed and heated at 80° C. for eight hours. The contents of the flask was then filtered. The residue was dissolved in water and extracted with ether. To the filtrate, 30 ml. of 10 *N* sodium hydroxide was added. The solution was distilled under reduced pressure in order to remove the solvent and the residue was cooled to 0° C. and made acid to Congo red with concentrated hydrochloric acid. A pale-yellow oil separated. This was removed and the residue was extracted with ether. These ether extracts and the oil were combined with the ether extracts of the filtered reaction mixture and dried over anhydrous sodium sulphate. The ether was evaporated, the product was distilled, b.p. 59° C. (24 mm.), 49° to 50° C. (17 mm.), and 2.37 gm. (30% yield) of a colourless oil was obtained, sp.gr.$^{20}_{20}$ 1.061, the purity of which was 100% by iodine titration. Calc. for $C_3H_8S_2$: C, 33.28; H, 7.45%. Found: C, 33.86; H, 7.60%.

A comparison of the boiling point and specific gravity of this compound with those of 1,2- and 1,3-dimercaptopropane made from the dihalogen compounds shows that it was the 1,2-isomer that was obtained in the above preparation. A molecule of hydrogen sulphide was thus introduced into an

unsaturated molecule by the action of ammonium hydrosulphide under pressure. The mode of addition was 'normal' according to an extension of Markownikoff's rule.

Preparation II. Sodium hydrosulphide was prepared from sodium hydroxide (80.0 gm.) in 1000 ml. of ethanol. 1,2-Dibromopropane (103.5 gm.) was added and the solution was heated at 25° C. for five days. The product was obtained by the addition of three volumes of water to the reaction mixture and was distilled under reduced pressure, b.p. 51° to 52° C. (17 mm.), sp. gr. $\frac{20}{20}$ 1.063, yield 32%, purity 97% by iodine titration. Calc. for $C_3H_8S_2$: C, 33.28; H, 7.45; S, 59.27%. Found: C, 33.16; H, 7.69; S, 58.68%.

1,3-Dimercaptopropane

Preparation I. 1,3-Dibromopropane (14.6 gm.) was added to 100 ml. of a suspension of ammonium hydrosulphide (15 gm.) in ethanol and the solution was heated at 70° C. for eight hours. The product obtained from half of the ethanol solution by formation of the sodium mercaptide weighed 1.55 gm. The product obtained from the other half of the ethanol solution by the addition of three volumes of water weighed 3.02 gm. The two samples of the product were combined and distilled under reduced pressure, b.p. 57° C. (12 mm.), sp.gr.$^{20}_{20}$ 1.080, yield 20%, purity 99.5% by iodine titration. Calc. for $C_3H_8S_2$: C, 33.28; H, 7.45%. Found: C, 33.88; H, 7.67%.

Preparation II. Sodium hydroxide (40.0 gm.) was converted to sodium hydrosulphide in 500 ml. of ethanol. 1,3-Dibromopropane (50.5 gm.) was added and the solution was heated at 30° C. for seven days. The thiol was obtained by the addition of three volumes of water to the ethanol solution and was distilled under reduced pressure, b.p. 94° C. (60 mm.), 64° to 65° C. (15 mm.), sp.gr.$^{20}_{20}$ 1.081, yield 53%, purity 96.5% by iodine titration. Calc. for $C_3H_8S_2$: C, 33.28; H, 7.45; S, 59.27%. Found: C, 33.64; H, 7.58; S, 59.69%.

1,2,3-Trimercaptopropane

Preparation I. 1,2,3-Tribromopropane (14.0 gm.) was added to 15 gm. of ammonium hydrosulphide in 100 ml. of ethanol and the mixture was heated at 60° C. for 12 hr. The thiol was obtained by the addition of three volumes of water to the ethanol solution and was distilled under reduced pressure and 0.72 gm. (10% yield) was obtained, purity 97% by iodine titration. Calc. for $C_3H_8S_3$: C, 25.68; H, 5.75%. Found: C, 26.18; H, 5.75%.

Preparation II. Sodium hydrosulphide was prepared from sodium hydroxide (80.0 gm.) in 1000 ml. of ethanol. 1,2,3-Tribromopropane (140 gm.) was added and the solution was heated at 60° C. for six hours. The product was obtained by the addition of three volumes of water to the reaction mixture and was distilled under reduced pressure, b.p. 60° C. (0.40 mm.), 54° C. (0.18 mm.), 50° to 52° C. (0.045 mm.), sp.gr.$^{20}_{20}$ 1.233, yield 43%, purity 98% by iodine titration. The product was difficult to distil. Calc. for $C_3H_8S_3$: C, 25.68; H, 5.75; S, 68.60%. Found: C, 25.51; H, 5.80; S, 67.76%.

1,2-Dimercapto-n-butane

Preparation I. Sodium metal (16.0 gm.) was dissolved in 400 ml. of ethanol and converted to sodium hydrosulphide and this was precipitated by the addition of ether. Sodium hydrosulphide (32 gm.) and 1,2-dichloro-*n*-butane (12.0 gm.) were added to 400 ml. of ethanol and heated at 80° C. for 12 hr. The thiol was obtained through the formation of the sodium mercaptide and was distilled under reduced pressure, b.p. 77° C. (28 mm.), 49° C. (5 mm.), sp.gr.$^{20}_{20}$ 1.039, yield 25%, purity 98% by iodine titration. Calc. for $C_4H_{10}S_2$: C, 39.27; H, 8.24%. Found: C, 39.30; H, 8.22%.

Preparation II. Sodium hydrosulphide was prepared from sodium hydroxide (40.0 gm.) in 500 ml. of ethanol. 1,2-Dichloro-*n*-butane (31.7 gm.) was added and the solution was heated at 25° to 30° C. for seven days. The thiol was obtained by the addition of three volumes of water to the reaction mixture and was distilled under reduced pressure, b.p. 58° C. (13 mm.), yield 22%. The product was purified by conversion to the sodium mercaptide and by repeated distillation. The purity was 96% by iodine titration. Calc. for $C_4H_{10}S_2$: C, 39.27; H, 8.24; S, 52.7%. Found: C, 39.31; H, 8.03; S, 51.65%.

2,2′-Dimercaptoethyl ether

Preparation I. 2,2′-Dichloroethyl ether (11.2 gm.) was added to 100 ml. of a suspension of ammonium hydrosulphide (16.0 gm.) in ethanol and the mixture was heated at 80° C. for 12 hr. The solvent was removed by evaporation under reduced pressure, and the product was extracted with ether and distilled under reduced pressure, b.p. 53° to 54° C. (0.05 mm.), sp.gr.$^{20}_{20}$ 1.127, yield 35%, purity 100% by iodine titration. Calc. for $C_4H_{10}OS_2$: C, 34.73; H, 7.29%. Found: C, 34.90; H, 7.32%.

Preparation II. Sodium hydrosulphide was prepared from sodium hydroxide (40.0 gm.) in 500 ml. of ethanol. 2,2′-Dichloroethyl ether (35.7 gm.) was added and the solution was heated at 30° C. for seven days. The solution was made acid to Congo red by the addition of hydrochloric acid, filtered, and the solvent was evaporated under reduced pressure. The product was extracted with ether and distilled under reduced pressure and 6.8 gm. of thiol (20% yield) was obtained. The compound was purified by formation of the sodium mercaptide. The purity was 99% by iodine titration. Calc. for $C_4H_{10}OS_2$: C, 34.73; H, 7.29; S, 46.39%. Found: C, 34.98; H, 7.42; S, 46.79%.

3,3′-Dimercaptopropyl ether

Ammonium hydrosulphide (14.8 gm.) and 3,3′-dichloropropyl ether (12.5 gm.) were heated in 100 ml. of ethanol at 70° C. for five hours. The thiol was obtained by the addition of three volumes of water to the ethanol solution and was distilled under reduced pressure, b.p. 62° to 64° C. (0.125 mm.), sp.gr.$^{20}_{20}$ 1.055, yield 47%, purity 100% by iodine titration. Calc. for $C_6H_{14}OS_2$: C, 43.32; H, 8.49; S, 38.59%. Found: C, 43.04; H, 8.31; S, 38.70%.

2,2'-Dimercaptoisopropyl ether

Sodium hydroxide (80.0 gm.) was dissolved in 1000 ml. of ethanol and converted to sodium hydrosulphide. 2,2'-Dichloroisopropyl ether (85.5 gm.) was added and the solution was heated at 30° C. for 14 days and at 60° C. for six hours. The product was separated by the addition of three volumes of water to the reaction mixture and was purified by formation of the sodium mercaptide and by distillation under reduced pressure, b.p. 52° to 54° C. (0.55 mm.), sp. gr. $^{20}_{20}$ 1.053, yield 11%, purity 99% by iodine titration. Calc. for $C_6H_{14}OS_2$: C, 43.32; H, 8.49; S, 38.59%. Found: C, 42.78; H, 8.37; S, 38.58%.

1,3-Dimercapto-2-propanol

Preparation I. 1,3-Dibromo-2-propanol (10.7 gm.) was added to 10 gm. of ammonium hydrosulphide in 100 ml. of ethanol and the mixture was heated at 80° C. for 12 hr. The product was obtained by evaporating the alcohol under reduced pressure and extracting the residue with ether. The compound was distilled under reduced pressure, b.p. 55° C. (0.040 mm.), yield 26%, purity 95% by iodine titration. Calc. for $C_3H_8OS_2$: C, 28.98; H, 6.49%. Found: C, 29.37; H, 6.25%.

Preparation II. Sodium metal (23.0 gm.) was dissolved in 400 ml. of ethanol and converted to sodium hydrosulphide. 1,3-Dibromo-2-propanol (54.5 gm.) was added and the solution was heated at 30° C. for 72 hr. and at 60° C. for two hours. Glacial acetic acid (60.0 gm.) was added and the solvent was removed by evaporation under reduced pressure. Water was added to dissolve the solid material and ammonium hydroxide was added until the solution was alkaline to litmus. The product was extracted with ether and distilled under reduced pressure, b.p. 60° C. (0.060 mm.), sp.gr. $^{20}_{20}$ 1.241, yield 40%, purity 95% by iodine titration. Calc. for $C_3H_8OS_2$: C, 28.98; H, 6.49; S, 51.63%. Found: C, 29.33; H, 6.58; S, 52.49%.

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SYNTHESIS OF AMINO ACIDS FROM SUBSTITUTED CYANOACETIC ESTERS. 4-ALKYL-3-AMINOPYRAZOLONES[1]

By Paul E. Gagnon[2], Kenneth Savard[3], Roger Gaudry[4], and Edwin M. Richardson[5]

Abstract

Four α-amino acids, namely, norleucine, isoleucine, methionine, and S-benzylhomocysteine, have been prepared from the corresponding alkylcyanoacetic esters. Methionine has been obtained in excellent yield.

The intermediate alkylcyanoacethydrazides formed 4-alkyl-3-aminopyrazolones. Four members of this new series, 4-*n*-butyl-, 4-*sec*-butyl-, 4-(β-methylthiolethyl)-, and 4-(β-benzylthiolethyl)-3-aminopyrazolones are described and their ultraviolet absorption spectra given.

The following compounds have also been isolated: ethyl α-cyano-γ-methylthiolbutyrate, ethyl α-cyano-γ-benzylthiolbutyrate, α-cyano-*n*-caprohydrazide, isopropylidene α-cyano-*n*-caprohydrazide, anisal α-cyano-β-methylvalerhydrazide, anisal α-cyano-γ-methylthiolbutyrhydrazide, and anisal α-cyano-γ-benzylthiolbutyrhydrazide.

Introduction

The application of the Curtius reaction to the synthesis of amino acids was first suggested by Darapsky and Hillers (10) in 1915 by their synthesis of glycine. It was not until 1936 that the proposed method received further attention when Darapsky (9), starting from alkylcyanoacetic esters, successfully prepared, with fairly good yields, α-amino-*n*-valeric acid, α-aminoisoamylacetic acid, and *dl*-leucine.

Later, Gagnon, Gaudry, and King (13) obtained, by the same method, α-amino-δ-phenoxyvaleric acid and the three natural amino acids, *dl*-tyrosine, *dl*-phenylalanine and *dl*-valine. The high yields (50 and 60%) obtained by these authors in the synthesis of the last two amino acids, together with the fact that ether groups are not affected by the conditions of the Darapsky method, indicated the possibility of wider applicability of the Curtius reaction to the synthesis of amino acids.

The present study was undertaken primarily to extend the Darapsky application of the Curtius reaction to the preparation of sulphur-containing amino acids, in particular methionine. Before this was begun, experience was gained by first preparing the two isomeric amino acids, norleucine (VII, R = *n*-butyl) and isoleucine (VII, R = *sec*-butyl); the method was then

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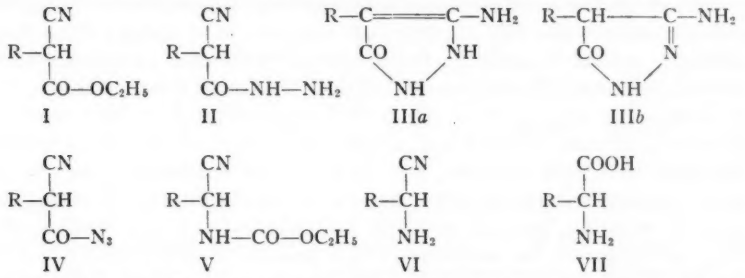
successfully applied to the synthesis of methionine (VII, R = β-methylthiolethyl) and S-benzylhomocysteine (VII, R = β-benzylthiolethyl).



I II IIIa IIIb

IV V VI VII

R = *n*-butyl, (CH_3-CH_2-CH_2-CH_2-); *s*-butyl, (CH_3-CH_2-$CH(CH_3)$-); β-methylthiolethyl, (CH_3-S-CH_2-CH_2-); β-benzylthiolethyl, (C_6H_5-CH_2-S-CH_2-CH_2-).

As starting materials, the alkylcyanoacetic esters (I) were prepared by the condensation of alkyl halides with ethyl cyanoacetate; when alkyl chlorides were employed, better yields were obtained by the addition of potassium iodide to the reaction medium (31). The formation of the hydrazides (II) took place quantitatively at room temperature; only one of the products, α-cyano-*n*-caprohydrazide (II, R = *n*-butyl) was obtained as a low melting solid, the remainder were viscous liquids and were identified by their crystalline condensation products with anisaldehyde.

The crude alkylcyanoacethydrazides, on standing for some time, underwent transformation to the corresponding 4-alkyl-3-aminopyrazolones (III*a*, III*b*), the rate of the reaction being increased when sodium hydroxide was used as a catalyst (14, 29).

Ultraviolet absorption determinations on these compounds revealed that the principal absorption peaks were in close agreement with those characteristic of the pyrazolone ring (3, 4, 25).

This is the first time that 3-aminopyrazolones substituted *only* in the 4-position are described. 3-Aminopyrazolones substituted in positions 1 and 2 have already been reported (26-30).

The conversion of the hydrazides through the azides (IV) to the urethanes (V) took place smoothly without loss. These intermediate products were viscous liquids and were not isolated. Hydrolysis of the urethanes, giving *dl*-norleucine in 45% yield and *dl*-isoleucine in 54% yield, was accomplished with aqueous hydrochloric acid (20%). The identification of the latter amino acid as the copper salt revealed the possibility that the two racemates, *dl*-isoleucine and *dl*-alloisoleucine, might be easily separated because of the difference in solubility of the copper salts; this point will be studied further and reported on in a subsequent publication.

The hydrolysis in 20% hydrochloric acid of the sulphur-containing urethane (V, R = β-methylthiolethyl) leading to methionine gave a very poor yield of the amino acid, and was accompanied by excessive tar formation. This

has previously been noted by Barger and Coyne (2) in the hydrolysis of the corresponding amino nitrile (VI). Although the choice of hydrolysing agents is limited by the instability of methionine towards sulphuric, hydrobromic, and hydriodic acids (5), a variety of reagents were tested; these included alcoholic sodium hydroxide, fused baryta (11), and anhydrous alcoholic hydrochloric acid followed by mild hydrolysis (imino ether method). Yields of methionine ranging from 12 to 45% were obtained. Best results were given by a mixture of formic acid, hydrochloric acid, and water. This reagent, described by Carter, Stevens, and Ney (6) and successfully used by others (23), completely dissolved the urethane at the boiling point, and, after six hours of refluxing, gave an excellent yield of *dl*-methionine corresponding to 85% conversion of the urethane; longer periods of refluxing (24 hr.) caused considerable destruction (43%) of the product. The yield of *dl*-methionine calculated from ethyl α-cyano-γ-methylthiolbutyrate (I, R = β-methylthiolethyl) was 73%, and is the highest reported so far in the various amino acid syntheses by the Darapsky method.

The mixture of formic acid, hydrochloric acid, and water was employed in the preparation of S-benzylhomocysteine.

Experimental*

Ethyl Alkylcyanoacetates (I)

To a warm solution of sodium ethoxide (0.6 mole of sodium and 350 to 500 ml. of absolute ethyl alcohol), freshly distilled ethyl cyanoacetate (1.2 moles) and later the alkyl chloride or bromide (0.6 mole) were added and the solution was refluxed on a water-bath for the required length of time. After the excess of alcohol had been removed by distillation, the residual sludge was poured into cold water. The dark red oily layer formed was separated and the aqueous layer extracted several times with ether. The oily layer and the ethereal extracts were combined, and the solution dried over anhydrous sodium sulphate. The ether was removed by distillation and the residue fractionated. The properties and yields of the ethyl alkylcyanoacetates are given in Table I.

Alkylcyanoacethydrazides (II)

The ethyl alkylcyanoacetate (0.06 mole) and aqueous hydrazine hydrate (85%, 0.06 mole) were mixed and dissolved in absolute ethyl alcohol. The solution was allowed to stand at room temperature overnight and then freed from solvent in a vacuum desiccator. The reaction was quantitative. The residual viscous products could not be induced to crystallize, except α-cyano-*n*-caprohydrazide, and were used without further purification. A small quantity of α-cyano-*n*-caprohydrazide was recrystallized from ether (below room temperature) and gave colourless crystals: m.p. 49° to 51° C. Calc. for $C_7H_{13}ON_3$: N, 27.10%. Found: N, 27.20%. The alkylcyanoacethydrazides were identified by condensation with acetone or anisaldehyde in the usual way. The properties of the products obtained are given in Table II.

**All melting points are uncorrected.*

TABLE I

ETHYL ALKYLCYANOACETATES, $RCH(CN)COOC_2H_5$

Alkyl group, R	Starting material	Time of reaction, hr.	Yield, %	B.p., ° C.	n_D^{25}
n-Butyl[a]	CH_3–CH_2–CH_2–CH_2–Br	20	76	93.5–96 (3.5 mm.)	1.4246
s-Butyl[b]	CH_3–CH_2–$CH(CH_3)$–Br	20	59	92.5–93.5 (5.0 mm.)	1.4267
β-Methylthiolethyl[c]	CH_3–S–CH_2–CH_2–Cl[d, e]	12	54	131–133 (5.0 mm.)	1.4725
β-Benzylthiolethyl[f]	C_6H_5–CH_2–S–CH_2–CH_2–Cl[d, g]	12	49	169–170 (0.5 mm.)	1.5331

[a]*Ref. (1, 15);* [b]*Ref. (1,8).* [c]*Calc. for $C_8H_{13}O_2NS$: N, 7.53; S, 17.22%. Found: N, 7.53; S, 17.10%.* [d]*Potassium iodide (2.0 gm.) added to the reaction mixture.* [e]*Prepared from β-hydroxyethyl methyl sulphide kindly supplied by Dr. Eric Stiller of Wyeth Inc. Philadelphia.* [f]*Calc. for $C_{14}H_{17}O_2NS$; N, 5.33; S, 12.20%. Found: N, 5.33; S, 12.00%.* [g]*Prepared according to the procedure of Patterson and du Vigneaud (22).*

TABLE II

CONDENSATION PRODUCTS OF ALKYLCYANOACETHYDRAZIDES

R′-CH(CN)-CO-NH-N=CR″R‴

R′	R″	R‴	Solvent	M.p., ° C.	Formula	Analysis, %			
						Nitrogen		Sulphur	
						Calc.	Found	Calc.	Found
n-Butyl	Methyl	Methyl	Ether	79– 80.5	$C_{10}H_{17}ON_3$	21.53	21.56	—	—
s-Butyl	H	*p*-Methoxyphenyl	EtOH	140–141	$C_{14}H_{19}O_2N_3$	15.39	15.41	—	—
β-Methylthiolethyl	H	*p*-Methoxyphenyl	MeOH	135–137	$C_{14}H_{17}O_2N_3S$	14.43	14.17	11.01	11.00
β-Benzylthiolethyl	H	*p*-Methoxyphenyl	MeOH	127–128	$C_{20}H_{21}O_2N_3S$	11.44	11.19	8.73	8.55

Amino Acids (VII)

The crude alkylcyanoacethydrazides were converted through the azides to the corresponding urethanes in the manner previously outlined (9, 13). The urethanes were viscous liquids obtained in quantitative yields except in the case of the urethane leading to S-benzylhomocysteine, where the yield was 26%. The hydrolysing medium was added to the crude urethane and the mixture refluxed. After filtration, the solution was evaporated to dryness *in vacuo*. The excess of acid was removed by repeatedly dissolving the residue in water and evaporating to dryness *in vacuo*. The residue was then dissolved in the minimum quantity of hot water and decolorized with charcoal (norite). The clear solution was adjusted to the isoelectric point of the amino acid with ammonium hydroxide, and the resulting precipitate dissolved by

heating to the boiling point. An equal volume of ethyl alcohol was added and the solution cooled for a few hours. Glistening crystals were formed. They were filtered, washed with cold water followed by alcohol, and dried. Concentration of the filtrate to small volume and treatment with alcohol yielded a second crop. Recrystallization from water–ethyl-alcohol yielded the pure product, which gave a positive ninhydrin test.

The properties and yields of the amino acids obtained by this procedure are listed in Table III, together with the properties of the derivatives prepared.

TABLE III

AMINO ACIDS

Compound	Hydrolysis medium	Time, hr.	Yield,[a] %	Formula	Nitrogen, %		Derivative
					Calc.	Found	
dl-Norleucine	20% HCl	22	45	$C_6H_{13}O_2N$	10.68	10.83	Hydantoin, m.p. 138° to 139°C.[b]
dl-Isoleucine	20% HCl	22	54	$C_6H_{13}O_2N$	10.68	10.91	Cu salt[c]
dl-S-Benzylhomocysteine[d]	Mixed acids[e]	10	9	$C_{11}H_{14}O_2NS$	6.22	5.98	——
dl-Methionine[f]	Mixed acids[e]	6 24	73 58	$C_5H_{11}O_2NS$[g]	9.39	9.31	Hydantoin, m.p. 115° to 117° C.[h]

[a]*Calculated from the ethyl alkylcyanoacetate.* [b]*Ref. (18).* [c]*Calc. for* $Cu(C_6H_{12}O_2N)_2$*; N, 8.66%. Found: methyl alcohol soluble salt, N, 8.48%; methyl alcohol insoluble salt, N, 8.66%.* [d]*M.p. 194° to 196° C.* [e]*Equal portions by volume of concentrated hydrochloric acid, 85% formic acid, and water.* [f]*McCarthy–Sullivan test (20) was positive.* [g]*Calc. S, 21.49%. Found: S, 21.23%.* [h]*Ref. (16).*

The copper salt of *dl*-isoleucine was prepared according to the method of Town (24). Extraction of the dry salt with methyl alcohol revealed the presence of two components, one very soluble in methyl alcohol and the other slightly soluble, probably *dl*-isoleucine and *dl*-alloisoleucine.

4-Alkyl-3-aminopyrazolones (III)

The crude alkylcyanoacethydrazides, treated with sodium hydroxide according to the method of Hepner and Fajersztejn (14), gave rise to the corresponding 4-alkyl-3-aminopyrazolones, which were difficult to separate from the sodium acetate present.

The crude alkylcyanoacethydrazides, on standing at room temperature for several weeks, *also* yielded pyrazolones quantitatively. These were more readily purified by recrystallization from ethyl acetate or a mixture of this solvent and ether. The resulting colourless products were identified by mixed melting point determinations and analysis. One sample, analysed by the ordinary Kjeldahl method, gave figures corresponding to only one nitrogen atom per mole, whereas after pretreatment with hydriodic acid (12), figures corresponding to the three nitrogen atoms were obtained. These 4-alkyl-3-

aminopyrazolones, the properties of which are listed in Table IV, were employed for the ultraviolet absorption determinations.

The absorption measurements were made on a Beckman Quartz Spectrophotometer Model DU (7), using a hydrogen discharge tube as light source. All measurements were made in stoppered quartz cells of 1.000 ± 0.002 cm. length. Redistilled absolute methyl alcohol was employed throughout the determinations and solutions were prepared by progressive dilution of a sample weighed on a microbalance. Initial measurements of each sample were made in the spectral range 4000 to 2200 Å, at intervals of 20 Å; in the region of the absorption maxima and minima, readings were made at intervals of 10 Å. Subsequent measurements were limited to the range 3200 to 2200 Å when it was apparent that the compounds studied possessed no significant absorption in the region above 3200 Å. A spectral band width of 10 Å was employed at all wave-lengths.

The values recorded in Table IV and Fig. 1 were calculated in terms of E_m, where E_m is molar extinction coefficient, defined by the equation

$$E_m = \frac{M}{c.d} \cdot \log_{10} I_0/I$$

M = the molecular weight of the solute,

c = the concentration of solute in milligrams per millilitre,

d = the cell thickness (length of light path) in cm.,

I_0 = the intensity of the incident light,

I = the intensity of the emergent light. The value of the extinction coefficient, I_0/I, is read directly from the photometer drum of the instrument.

A close inspection of the curves shown in Fig. 1 reveals the fact that the spectra of 4-(β-methylthiolethyl)-3-aminopyrazolone and 4-(β-benzylthiolethyl)-3-aminopyrazolone are almost identical, the slight deviation of the latter being due to the benzene ring which, 'insulated' from the conjugated system, contributes additively to the absorption (17). The secondary maxima present in the spectra of 4-*n*-butyl-3-aminopyrazolone and, to a lesser degree (as an inflection point only), 4-*sec*-butyl-3-aminopyrazolone is in the region of absorption of α, β-unsaturated ketones (17, 32); this would indicate that at least under the conditions of the measurements these compounds exist in both structural forms III*a* and III*b*. A further indication is the melting point range of several of these products. Biquard and Grammaticakis, by preparing 1-phenyl-2,3,4-trimethylpyrazolone, which can exist only as the form corresponding to III*a*, and 1-phenyl-3,4,4-trimethylpyrazolone corresponding to structure III*b*, have shown that pyrazolones having these two structures exhibit distinctly different absorption spectra (4). As a result of their work, it may be concluded that in alcoholic solution, 4-*n*-butyl- and 4-*sec*-butyl-3-aminopyrazolones exist as both structural forms III*a* and III*b*, while 4-(β-methylthiolethyl)- and 4-(β-benzylthiolethyl)-3-aminopyrazolones exist solely as structural form III*b*.

TABLE IV

4-ALKYL-3-AMINOPYRAZOLONES

3-Aminopyrazolones	Formula	M.p., °C.	Mol. wt.[a]		Analysis, %								Ultraviolet absorption maxima	
					Carbon		Hydrogen		Nitrogen[b]		Sulphur			
			Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found	Å	E_m
4-*n*-Butyl	$C_7H_{13}ON_3$	128–130	155	158	—	—	—	—	27.10	26.94	—	—	2480[c] 2820[c]	8450 3620
4-*s*-Butyl	$C_7H_{13}ON_3$	145–146	155	157	54.15	54.45	8.44	8.42	27.10	26.75	—	—	2480[c] (2720)[d]	4750 3520
4-(β-Methylthiolethyl)	$C_6H_{11}ON_3S$	121–122	173	197	41.58	41.43	6.40	6.00	24.27	24.36	18.51	18.61	2450[c]	13100
4-(β-Benzylthiolethyl)	$C_{12}H_{15}ON_3S$	109–111	249	208	—	—	—	—	16.87	16.95	12.86	12.59	2450	13000

[a]In acetone, method of Menzies and Wright (21). [b]Hydriodic acid digestion prior to Kjeldahl determination (12). [c]Absorption spectra of product prepared with sodium hydroxide was identical. [d]Inflection point.

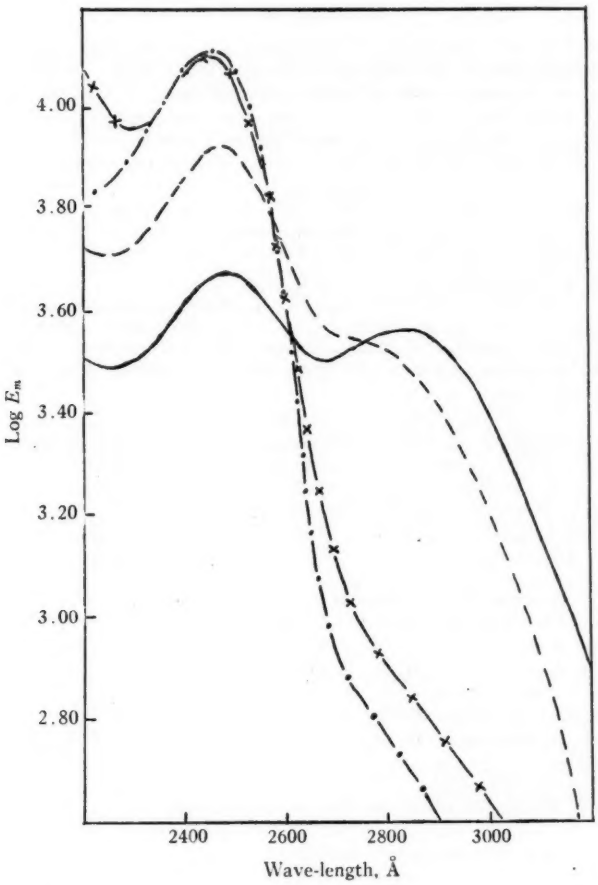


FIG. 1.

——•——•—— *4-n-Butyl-3-aminopyrazolone*
— — — — — — — — *4-s-Butyl-3-aminopyrazolone*
— · —— · —— *4-(β-Methylthiolethyl)-3-aminopyrazolone*
——×——×—— *4-(β-Benzylthiolethyl)-3-aminopyrazolone*

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THE REACTION OF β,β′-DICHLORODIETHYL SULPHIDE WITH PROTEINS[1]

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Abstract

The reaction of β,β′-dichlorodiethyl sulphide with various proteins and their constituents has been tested under relatively mild conditions. Most reactions have been carried out in aqueous media at 25° C., usually in the presence of sodium chloride. The rate of disappearance of mustard has been followed iodimetrically as an index of the reaction.

No reaction could be demonstrated with the prolamins, zein and gliadin, in ethanol. There was a definite reaction with keratin of human skin in which a 24% increment in total sulphur occurred and a more doubtful one with keratein from human hair.

The most notable reaction took place with yeast and thymus nucleoproteins in which increases of 250 and 156% in sulphur occurred, respectively. Combination of mustard with both the protein and nucleic acid portions was demonstrated. The extent of these reactions is indicated in terms of milligrams of mustard per gram of substrate as follows: yeast nucleoprotein, 216; thymus nucleoprotein, 47; salmine, 47; globin, 22; yeast nucleic acid, 126. The purines, adenine and guanine, were found to combine readily with mustard at 25° C., forming thiazans. No reaction could be demonstrated with uracil or thiamine.

These experiments have led us to the conclusion that one of the major effects of mustard in its physiological action is the combination and precipitation of the nucleoproteins of the cell, thus inhibiting natural processes of cellular regeneration.

The purpose of the investigation to be described was to analyse the reaction between β,β′-dichlorodiethyl sulphide and various types of proteins. It was hoped to discover something of the kinetics of the reaction, especially as applied to the proteins of the skin, to prepare reaction products and to obtain information as to the nature of the reaction, as a guide to a better understanding of the mechanism of action of mustard on human skin.

Previous investigations have established reactions with many amino acids (5, 16). These reactions resulted in α–N-substitution products, similar to those with amines. Evidence for a reaction with proteins has been based largely on the formation of a product with increased sulphur content (3) or on specific immunological properties. In a few instances the disappearance of reactive radicals in the protein molecule, such as α-amino, free carboxyl, sulphhydryl and phenolic, has been measured as an index of the reaction or the stability of the compound formed.

Experimental

This work began in February 1942, and was concluded in July 1945. It has involved experiments with prolamins, keratins from human skin and

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hair, yeast and thymus nucleoproteins and their constituent parts, including salmine, nucleic acid, adenine, guanine and uracil, and in addition a few observations on globin and thiamine. After the development for our purposes of a satisfactory quantitative method for the estimation of mustard, the general procedure has been essentially the same.

General Procedure

The substrate was dissolved, completely when possible, usually in an aqueous medium in low concentration. The reaction was carried on in a large water-bath maintained at 25° C. ± 1°. The reaction mixture was stirred mechanically at such a speed as not to incorporate any air in the liquid. The medium was maintained between pH 7 and 8, determined electrometrically, by the addition of sodium bicarbonate, carbonate, or hydroxide as required. Mustard was added in small quantities as a 10% solution in absolute ethanol, at such intervals as was indicated by the determination of free mustard in the reaction mixture. The rate of disappearance of mustard was followed by iodimetric titration as given below. When the reaction appeared complete the substrate was precipitated, fractionated, purified carefully, dehydrated, and finally desiccated over phosphorus pentoxide at 61° C. in a pistol desiccator. Microchemical elementary analysis for sulphur has been used as confirmatory evidence of reaction.

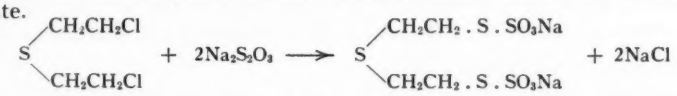
In many instances sodium chloride was added to the medium at 3 to 5% concentration to depress the rate of hydrolysis of mustard (19). We have found the rate depressed to about one-eighth of that in distilled water under our experimental conditions. The only disadvantage to this procedure is the subsequent difficulty in separation if the reaction product is readily soluble.

Microanalysis of sulphur was done by catalytic combustion followed by gravimetric estimation as barium sulphate. The nitrogen was determined either by micro-Dumas or by digestion with hydriodic acid under pressure at 200° C. for two hours, followed by micro-Kjeldahl estimation.

All results are expressed on an ash-free basis.

Iodimetric Estimation of Mustard

At intervals during the reaction, 1 ml. of the reaction mixture was pipetted into a 50 ml. Erlenmeyer flask, mixed with 2 ml. of sodium thiosulphate (0.01 *N*) and heated for 10 min. at 60° C. The excess thiosulphate was determined by titration with 0.01 *N* iodine in a microburette, with 0.5% soluble starch as indicator, in a neutral or slightly acid medium. The method depends upon the reaction of any free mustard with sodium thiosulphate to form sodium dithiosulphatyl diethyl sulphide and titration of excess thiosulphate.



In this method it was necessary to rule out by control test any reaction of the iodine or thiosulphate with the substrate. It was found that 1 ml. of thiosul-

phate (0.01 *N*) was equivalent to 0.804 mgm. of mustard. Theoretically it should have reacted with 0.795 mgm. The method was reproducible to ± 10% approximately.

Materials

Two samples of mustard were used in the course of this investigation. One, obtained from Dr. George Wright, University of Toronto, had a slight yellowish colour and a melting point of 12.3° C. as against the usually accepted figure of 13° to 14° C. (20). The other, supplied by Dr. Robert MacIntosh, McGill University, melted at 12.4° C.

The methods of preparation or purification of the various substrates will be described in their separate sections below.

Prolamins

Zein

Zein was chosen first as substrate because of its solubility in common with mustard in ethanol and because of the slow rate of hydrolysis of mustard under these conditions. A commercial sample of zein (Pfanstiehl) was purified by a modification of the method of Osborne and Clapp (18) and of Dill and Alsberg (9). Zein (3.135 gm.) in 100 ml. of ethanol (90% *v/v*) was treated with 0.50 ml. of mustard (≈ 0.637 gm.) and 1.00 gm. of sodium bicarbonate and the mixture heated at 60° C. for 18 hr. A blank was first run in the same manner to determine the rate of hydrolysis of mustard under these conditions. The results are shown in Table I. It is evident that decomposition in 90% ethanol at 20° C. is slow but is almost complete in 24 hr. at 60° C.

TABLE I

RATE OF HYDROLYSIS OF MUSTARD IN 90% ETHANOL

Time	Temp., ° C.	Hydrolysis, %
6 days	20	14.6
8 hr.	60	61.2
16 hr.	60	79.3
25 hr.	60	94.0

The H^+ ion concentration changed from pH 8.52 to 2.12. Excess mustard was removed by petrol extraction and the reaction product was isolated and purified exhaustively. On microanalysis the following values were obtained: 1.05% S and 15.9% N in control and 1.05% S and 10.9% N in the reaction product.

There would thus seem to be no evidence of combination between zein and mustard despite the time available for a reaction. The loss in total nitrogen is appreciable and may be explained by hydrolysis of amide linkages during the course of the reaction. Zein is remarkable because of its very low content of the basic amino acids. It possesses, however, a high content of dicarboxylic

acids but the carboxyl radicals appear to be in amide linkage. Cohn, Berggren, and Hendry (6) remark that "the extremely small concentration both of acid and basic groups, and the very low solubility of zein in water, and in dilute solutions of acids and alkalis characterize zein as a very inert protein, more inert than any other that we have thus far investigated."

The action of an alcoholic solution of the reaction product was tested by application of a concentrated solution to the skin of very young mice. It induced a very slight erythema without induration, so that the result was considered negative.

Gliadin

A sample was prepared by the method of Osborne and Harris as modified by Dill and Alsberg (9) and Dill (8). Gliadin (3.009 gm.) was dissolved in 100 ml. of ethanol (80% *v/v*), and 0.5 ml. of mustard (≃ 0.637 gm.) added and 1.00 gm. of sodium bicarbonate. The reaction mixture was heated in an all-glass apparatus under reflux at 60° C. for 23 hr. A control without the gliadin was also carried out as a guide to the rate of hydrolysis of mustard under these conditions. The results are given in Table II.

TABLE II

RATE OF REACTION IN .80% ETHANOL AT 60° C.

Control		Gliadin	
Time, hr.	Hydrolysis, %	Time, hr.	Hydrolysis, %
2.0	44.1	2.0	48.8
4.2	55.9	5.0	72.2
7.2	81.0	7.0	76.8
15.2	97.6	12.0	81.4
—	—	23.0	100.0

The pH was 8.96 at the start and 9.28 at the end of the reaction. The reaction product was isolated and purified by repeated precipitation in absolute acetone and resolution in 70% ethanol. The microanalyses showed 1.37% S and 17.9% N in the control and 1.28% S and 17.2% N in the reaction product.

As with zein there is no evidence of a combination of gliadin with mustard on the basis of an increase in the sulphur content. Gliadin possesses few diamino acids but has a high content of glutamic acid (43.77%) in amide linkage (amide N, 5.2%). It is probable that the prolamins do not possess the requisite radicals, such as the sulphhydryl, to react with mustard, or that an aqueous medium is required.

Keratins

A closer approach to physiological conditions was therefore attempted. Several specimens of human skin from abdomen and leg were defatted, dried,

and comminuted in a Wiley mill to pass a No. 20 mesh sieve. A white fibrous powder was obtained.

A sample of human hair was washed with water, defatted with ether, and converted into keratein by way of increasing the available sulphhydryl groupings and the solubility. The product was a light grayish powder.

Keratin from Human Skin

A portion (1 gm.) of the mixed proteins of human skin was suspended in 200 ml. of sodium carbonate (0.1 *M*), and 0.5 ml. of mustard was added to the reaction mixture during a period of three hours, at 25° C. The solid remaining was washed thoroughly with 0.1 *M* sodium carbonate, water, aqueous acetone (50%), and absolute acetone. It was finally extracted with acetone in a Soxhlet apparatus for 48 hr. Results of microanalysis are given in Table III.

TABLE III

ANALYSES OF SKIN PROTEINS

—		I, %	II, %	Mean, %
Skin proteins (control)	S	0.38	0.53	0.46
	N	15.15	15.21	15.18
	Ash	1.46	1.37	1.42
Skin proteins–mustard	S	0.49	0.65	0.57
	N	15.49	15.68	15.59
	Ash	0.42	0.38	0.40

The increase in the mean for the sulphur content was therefore 0.11, or 24% over the content of the control as indication of mustard fixation. This is equivalent to 5.5 mgm. per gm. of keratin. The discrepancies in the duplicates should, however, be taken into consideration.

Keratin from Human Hair

Washed defatted hair (50 gm.) was treated with sodium thioglycolate at pH 12 according to the procedure of Goddard and Michaelis (11). It was dissolved in 0.1% sodium hydroxide and precipitated with acetic acid. The keratein was washed with absolute ethanol and then absolute ether. Finally it was dried in a vacuum desiccator over calcium chloride at 37° C. for two days. This material was ground in a Wiley mill to pass through a No. 40 mesh sieve. A light gray powder (5 gm.) was obtained. The material was free from thioglycolic acid as shown by the cobalt test. It had an ash content of 1.6%.

A portion (1.0 gm.) was dissolved in 200 ml. of 0.1 *M* sodium carbonate and treated at 25° C. with 1.2 ml. of mustard dissolved in 25 ml. of absolute ethanol, added as 0.1 ml. at 30-min. intervals over a period of six hours. During the reaction the pH dropped from 10 to 8. A precipitate appeared in about three hours. This was centrifuged down, washed with acetone and

thoroughly dried. Microanalyses showed 4.64% S in the control and 4.82% S in the reaction product. The increment of sulphur was thus 0.18, equivalent to 10 mgm. of mustard per gm. of protein.

Nucleoproteins

Yeast Nucleoprotein

A specimen was prepared from fresh commercial baker's yeast by extracting with distilled water, 0.5% sodium carbonate, and 1 *M* sodium chloride. On acidification of the extracts the nucleoprotein precipitated at pH 4.0 from distilled water, at 5.7 from the carbonate extract, and at 4.7 from the saline extract. The precipitates were combined and dissolved in dilute ammonia at pH 8, reprecipitated and dried.

A portion (1 gm.) of the dried precipitate was dissolved in 0.1 *M* sodium bicarbonate at pH 8. To this was added a total of 6 ml. of mustard in absolute ethanol over a period of six hours. A precipitate, which proved to be insoluble in dilute acid and base, appeared during the second hour. It was thoroughly extracted before drying and analysis (Table IV).

TABLE IV

ANALYSES OF YEAST NUCLEOPROTEINS

—		I, %	II, %	Mean, %
Nucleoprotein (control)	S	1.68	1.76	1.72
	N	9.18	9.56	9.37
	Ash	7.48	7.69	7.59
Nucleoprotein–mustard	S	5.99	6.07	6.04
	N	10.40	10.77	10.19
	Ash	0.51	0.42	0.47

The results of the analysis show a marked increase of 250% in the sulphur content of the treated specimen. It is equivalent to the reaction of 216 mgm. per gm. of nucleoprotein.

Thymus Nucleoprotein

A sample of nucleoprotein from desiccated thymus (Armour) was prepared by the method of Mirsky and Pollister (17).

A portion (1.4 gm.) dissolved in 5.8% sodium chloride (1 *M*) was exposed to the action of 0.4 gm. of mustard at pH 7-8 and 25° C. for 11 hr. A precipitate appeared in 1.5 hr. Mustard was added about every 30 min. as it disappeared from solution. The precipitate was centrifuged off, washed in aqueous acetone, and dehydrated in absolute acetone. A second fraction settled out after dilution from the original reaction mixture on standing. This was dehydrated in ethanol. Microanalyses for sulphur are given in Table V.

TABLE V

ANALYSES OF THYMUS NUCLEOPROTEINS

—	I, %	II, %	Mean, %
Thymus nucleoprotein (control)	0.56	0.65	0.61
Nucleoprotein–mustard, 1st fraction	1.65	1.47	1.56
Nucleoprotein–mustard, 2nd fraction	1.67	1.45	1.55

The ash contents were 7.09, 7.66, and 4.7%, respectively. The two fractions isolated were taken to be the same compound. *There is a marked increase in bound sulphur between treated and untreated specimens of 0.95 or 156%, comparable with the reaction noted with yeast nucleoprotein.* This is equivalent to 4.7 mgm. of mustard per gm. of protein.

Constituents of Nucleoprotein

Our attention was next directed to an analysis of the reaction with the various constituents of nucleoprotein. To this end we have studied the effect of mustard on globin, salmine, nucleic acid, and finally purines and pyrimidines.

Globin

The method of preparation from dog's blood was that of Anson and Mirsky (1) and the product was a dark brown powder, soluble in aqueous solvents. The reaction was carried out with 0.5 gm. dissolved in 200 ml. of 3% sodium chloride acidified with acetic acid to pH 3, and two additions of 0.25 ml. of mustard over a period of 11 hr. The reaction product formed a precipitate on the addition of sodium hydroxide. This was dispersed readily in aqueous acetone and reprecipitated only under the influence of sodium chloride. It was washed in water and dehydrated by absolute ethanol. The following were the results on microanalysis: 0.67% S and 15.8% N in the control and 1.12% S and 15.3% N in the reaction product.

The reaction product isolated had a very high ash content which would reduce the accuracy of the estimation of total sulphur. However, there appeared to be a definite increment in total sulphur equivalent to 22 mgm. of mustard per gm. of protein. No further experiments were done with globin.

Salmine

A few grams of salmine sulphate (obtained from Connaught Laboratories, through the courtesy of Dr. D. A. Scott) was dissolved in water and all sulphate precipitated with a solution of barium chloride, leaving salmine chloride in solution. The free salmine was precipitated by adjusting the pH to 8.5 and adding ethanol (95%). The product was a white powder.

Salmine hydrochloride (1.5 gm.) was dissolved in 120 ml. of distilled water and treated with 0.8 gm. of mustard at 25° C. over a period of 30 hr. The pH was maintained at 8.0. The resulting solution was evaporated to small

volume *in vacuo* and six volumes of acetone added. The flocculent precipitate settled out as an oil. Solution and precipitation were repeated and the product dried in the pistol desiccator to a light brownish powder. On microanalysis the following results were obtained: 0.00% S in control and 0.95% S in the reaction product.

There was therefore an increase of 0.95% of sulphur, equivalent to 47 mgm. of mustard per gm. of protein. Assuming a molecular weight of 3000 for salmine, 1 mole of mustard per mole of protein would be equivalent to 53 mgm. per gm., which corresponds with the result obtained.

Nucleic Acid

Yeast nucleic acid (Eastman) (10 gm.) was purified after the procedure of Baumann (2) and Steudel and Izumi (21). The nucleic acid precipitated between pH 3 and 1.5 at 0° C. and was dehydrated with absolute ethanol and ether, yielding 6.8 gm. The biuret test was negative.

The reaction with mustard was carried out at 25° C. in 100 ml. of a solution containing 5% sodium chloride, 1% sodium bicarbonate, and 1% nucleic acid, over a period of six hours. Mustard was added twice as 0.25 ml. The initial pH was 8.20 and the final 8.15. The product was isolated in two fractions after extraction with petrol (i) precipitated at pH 4 to 1.4, and (ii) precipitated at 1.4 with ethanol. The precipitates were dehydrated with ethanol and acetone. The first fraction was a slightly yellow solid resembling the original. The second was a snow-white powder. The microanalytical results are given in Table VI.

TABLE VI

ANALYSES OF YEAST NUCLEIC ACID

—		I, %	II, %	Mean, %
Nucleic acid (control)	S	0	0	0
Nucleic acid–H (1st)	S	2.45	2.60	2.52
	Ash	21.6	20.9	21.3
Nucleic acid–H (2nd)	S	0.77	0.61	0.69
	Ash	22.2	21.3	21.8

There was thus an appreciable fixation of sulphur in the first fraction, comparable with the result obtained with yeast nucleoprotein, and much higher than that with globin. This is equivalent to 126 mgm. of mustard per gm. of product. These results led us to try the effect of mustard on adenine and guanine.

Guanine

A specimen of guanine was prepared according to the method of Hunter and Hlynka (13) from yeast nucleic acid. Owing to its slight solubility it was necessary to alter the usual procedure.

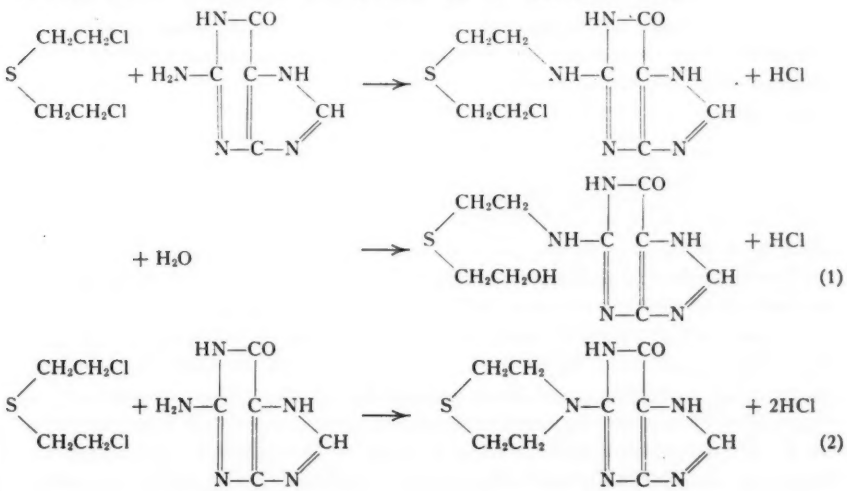
Guanine (0.5 gm.) was dissolved in 1000 ml. of 0.1% sodium hydroxide at 45° C. and 0.2 ml. of mustard in ethanol was added at intervals of 30 min. up

to a total of 2.5 ml. The pH was maintained between 7.5 and 8.0 during a reaction lasting about 10 hr. There was evidence by iodimetric titration of the rapid disappearance of mustard during the first few hours.

The reaction mixtures were neutralized with hydrochloric acid and concentrated *in vacuo* at 45° C. The residue was extracted repeatedly with benzine and finally the mixture was evaporated to dryness *in vacuo*. The slightly yellowish residue was removed from the flask with a small amount of water. A deep yellow solution and a white solid resulted. The solution was evaporated *in vacuo* until sodium chloride crystals separated out. The clear yellow mother liquor was evaporated to dryness and the residue extracted with 95% ethanol. The extract was treated with an excess of redistilled ether. A white precipitate, which turned yellow on standing and proved very hygroscopic (G-2), was obtained. It was examined microscopically and tested for sodium chloride but none was found present.

The insoluble portion from the reaction mixture was redissolved in water made alkaline (pH 8). This solution was concentrated and treated with excess of 95% ethanol to the formation of a white precipitate. This was next taken up in absolute ethanol and precipitated with anhydrous ether. The precipitate was very hygroscopic and had an ash content of 54.8% (G-3). The microanalytical results for total sulphur were as follows, calculated on an ash-free basis: (G-2) 11.7% S and (G-3) 11.8% S.

It was concluded from these results that a definite reaction had taken place and that the two fractions isolated were the same compound. The product differed from guanine in its greater solubility in water and alcohol, and its crystalline form. The most probable combination would be with the amino group in position 2. If the following reactions had taken place the products would have had a content of sulphur of (1) 12.5%, (2) 13.5%.



Owing to the hygroscopic character of the products obtained it is not possible to decide which reaction took place, but the probable character of the reaction is indicated by the agreement in the sulphur content, calculated and determined. This is in contrast with any further fixation of mustard molecules by the guanine, such as on positions 1 or 7, to give products with even higher content of sulphur.

Adenine

A specimen of adenine was prepared according to the method of Hunter and Hlynka (13) from yeast nucleic acid along with the guanine.

Adenine (0.4 gm.) was dissolved in 250 ml. of 0.25% acetic acid and 6 ml. of mustard added over a period of 16 hr. A constant pH of about 6 was maintained by adding small amounts of dilute sodium hydroxide. The reaction mixture was concentrated *in vacuo* and a precipitate formed. It was dissolved in water and reprecipitated by adding one volume of ethanol and two volumes of ether. The material had an ash content of 36.1% but possessed sufficient sulphur, 0.32%, to indicate that a reaction with adenine had taken place although not as rapidly or completely as with guanine.

Uracil

Owing to the limited solubility of uracil, a specimen of potassium uracil was prepared by the procedure of Johnson and Clapp (14) from uracil made from urea and malic acid by the synthesis of Davidson and Baudisch (7).

Uracil (1 gm.), dissolved in 200 ml. of 5% sodium chloride, was treated with 0.4 ml. of mustard added in small amounts over a period of six hours at 25° C. The initial pH of 9.45 fell to 7.8 during this interval. The residual mustard was allowed to hydrolyse and the solution was concentrated *in vacuo* to dryness. The residue was extracted with 95% ethanol, and the extract evaporated to dryness, and washed with absolute ethanol. There was no evidence of a reaction iodimetrically and the product obtained contained no sulphur. This is perhaps not surprising owing to the absence of any amino group in the pyrimidine molecule.

Cytosine

The result with uracil led us to attempt a reaction with cytosine. One effort of synthesis from uracil by the method of Hilbert and Johnson (12) was unsuccessful owing to the small yield obtained.

Thiamine

The biological importance of thiamine and its pyrimidine character suggested a trial of the reaction of this substance with mustard.

Thiamine hydrochloride (Merck) (1 gm.) was dissolved in 60 ml. of water and treated with 0.2 ml. of mustard at 25° C. for six hours; pH 7.0 was maintained by additions of dilute sodium hydroxide. The reaction product was isolated by adding equal volumes of alcohol and ether. It was redissolved in alcohol and again precipitated with ether. No evidence of a reaction was apparent iodimetrically and the sulphur analysis of the white crystalline

precipitate showed a value of 6.11% S in contrast to the control at 9.32% S. The value for sulphur in pure thiamine is 9.53%. There was thus no evidence of a combination with mustard on the free amino group and the product was either impure or some decomposition of the original thiamine must have taken place.

Discussion

A summary of our results is presented in Table VII. It is apparent from all recent investigations that mustard is a highly reactive substance and capable of combination with amino acids and proteins, under more or less

TABLE VII

SUMMARY

Substrate	Sulphur, %			Ratio mustard : product, mgm. : gm.
	Initial	Final	Increment	
Zein	1.05	1.05	0.00	0
Gliadin	1.37	1.28	− 0.09	0
Keratin (skin)	0.46	0.57	+0.11	5.5
Keratein (hair)	4.64	4.82	+ 0.18	10
Nucleoprotein (yeast)	1.72	6.04	+ 4.32	216
Nucleoprotein (thymus)	0.61	1.56	+ 0.95	47
Globin	0.67	1.12	+ 0.45	22
Salmine	0.00	0.95	+ 0.95	47
Nucleic acid	0.00	2.52	+ 2.52	126
Guanine	0.00	11.73	+ 11.73	586
Adenine	0.00	0.32	+ 0.32	16

physiological conditions. Our work has shown that a greater amount of mustard is combined with nucleoprotein than with any other protein. We show further that this reaction is applicable to both cytoplasmic and nuclear nucleoproteins but especially to the former to the extent of a combination of 216 mgm. per gm. of protein. Further analysis of this reaction has established that both the protein and nucleic acid fractions are able to combine with mustard. With nucleic acid the reaction is monomolecular, equivalent to 126 mgm. of mustard per gm. of nucleic acid (theoretical, 111, to molecular weight of product of 1304). The reactive constituent of nucleic acid is shown here to be guanine with which mustard combines in equimolar proportions (S found 11.8% as against a theoretical 12.5%). It is not shown, however, that a similar reaction does take place with adenine or cytosine. The reaction would most probably occur by hydrogen substitution on the free amino radical. No reaction took place with thiamine, however, under our conditions.

We have also shown that an equimolar combination between mustard and sa'mine takes place based on an assumed molecular weight of 3000 (47 mgm. of mustard per gm. of protein found, as against 53 mgm. theoretical). From these experiments with nucleoprotein, nucleic acid, and guanine, it would

appear that more mustard molecules are bound to the protein portion than to the nucleic acid portion of the molecule.

Recent publications have shown the protein nature of hexokinase (15) and the role of guanine as a coenzyme in the phosphorylation of glucose in the hexokinase reaction (4). Dixon and Needham (10) have explained the vesicant action of mustard on the basis of the inhibition of this reaction. It is possible therefore that the effect may be due to a reaction of mustard with guanine as shown above.

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5-HYDROXYQUINOLINE-8-CARBOXYLIC ACID—A COLORIMETRIC REAGENT FOR RUTHENIUM[1]

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Abstract

5-Aminoquinoline-8-carboxylic acid was prepared from 8-methylquinoline by nitration in the 5-position, oxidation of the methyl group to carboxyl, and reduction of the nitro group to amino. The 5-aminoquinoline-8-carboxylic acid was converted to 5-hydroxyquinoline-8-carboxylic acid through the diazonium salt. Some of the properties of the two new compounds were determined.

A transmission curve was established for solutions showing the colour developed by the reagent and ruthenium. The standard ruthenium solution used for this purpose contained the metal as it is obtained when distilled as the tetroxide and collected in hydrogen peroxide. Ruthenium in a solution as ammonium chlororuthenate could be determined from the same curve. One γ (1×10^{-6} gm.) of ruthenium in 50 ml. of solution may be quantitatively determined.

Introduction

Quinoline-8-carboxylic acid, a potential organic analytical reagent, has apparently not been extensively investigated except by Majumdar, who has published a paper concerning its use as a gravimetric reagent for copper (3) and has done some further work which will be published shortly (private communication). From preliminary tests in these laboratories it did not seem to be promising as a colorimetric reagent for metal ions, and it was decided to modify the nature of the compound by substitution and to examine the possibilities of the derivatives. The present paper deals with the synthesis of the 5-hydroxy derivative and its applicability as a colorimetric reagent for ruthenium, an element for which there has been no method of analysis by colorimetry available.

Part I

PREPARATION AND PROPERTIES OF 5-AMINOQUINOLINE-8-CARBOXYLIC ACID AND 5-HYDROXYQUINOLINE-8-CARBOXYLIC ACID

These compounds are not mentioned in the literature. 5-Nitroquinoline-8-carboxylic acid, which could easily be converted into 5-aminoquinoline-8-carboxylic acid, was prepared by Howitz and Nöther (2) from 8-methylquinoline by a series of five reactions, one of the intermediates being a very active vesicant. The authors have repeated these reactions successfully, but the over-all yield is very small (about 5% of theoretical) and the time required was considerable. Therefore another method of obtaining the acid from 8-methylquinoline had to be devised.

[1] *Manuscript received July 3, 1946.*

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Experimental

1. 5-Nitro-8-methylquinoline

This was prepared from 8-methylquinoline by the method of Noelting and Trautmann (4).

2. 5-Nitroquinoline-8-carboxylic acid

Thirty grams of 5-nitro-8-methylquinoline, 66 gm. of sodium bichromate dihydrate, and 150 ml. of water were boiled under a reflux condenser for five minutes. Ninety millilitres of concentrated sulphuric acid (sp. gr. 1.84) was added slowly to the boiling mixture, through the condenser. Care had to be taken to avoid violent spattering. The reddish-black mixture was refluxed for one hour, the colour changing to green. The solution was diluted to two litres with water, cooled, and 1 : 1 ammonium hydroxide was added until the greenish-brown chromium hydroxide began to precipitate. About 15 ml. of 6 *N* sulphuric acid was then added and the precipitate was filtered off and washed with four litres of water. The precipitate was suspended in 600 ml. of 1.5% sodium hydroxide solution and agitated violently for 15 min. The insoluble unchanged base was filtered off. The filtrate was neutralized with 6 *N* sulphuric acid, precipitating the white 5-nitroquinoline-8-carboxylic acid, which was filtered off. M.p. 209° to 213° C.* Net yield, 60% of theoretical.

3. 5-Aminoquinoline-8-carboxylic acid

An adaptation of the method of Dikshoorn (1) was used. Ten grams of 5-nitroquinoline-8-carboxylic acid and 100 ml. of 50% by volume aqueous acetic acid were boiled under a reflux condenser for five minutes. The solution was then cooled to 80° C., forming a semisolid white mass to which was added 8 gm. of iron powder. There was violent effervescence and the mass turned red. The reaction mixture was refluxed for half an hour, then shaken with 400 ml. of water and heated to 50° C. to dissolve the iron acetate. The solution was cooled and filtered. The filtrate was acidified with sulphuric acid, precipitating as the sulphate any amine that had dissolved. The precipitates of iron and the amine were separated from each other by recrystallization from 3½ litres of boiling water; yield, 60% of theoretical.

The amine formed long yellow needles, m.p. 271° to 272° C.* with decomposition, only slightly soluble in ethanol, benzene, cold water, dilute mineral acids (forming salts), soluble in boiling water and acetic acid. Found: C, 63.90, H, 4.21, N, 15.62%. Calc. for $C_{10}H_8N_2O_2$: C, 63.81, H, 4.28, N, 14.89%. The amine sulphate is a bright red solid, m.p. 244° C.* with decomposition, insoluble in dilute mineral acids, but soluble in concentrated mineral acids. The use of the amine as a reagent would be complicated by its insolubility in neutral and weakly acid solutions.

4. 5-Hydroxyquinoline-8-carboxylic acid

A solution of 5.7 gm. of 5-aminoquinoline-8-carboxylic acid, 5.7 gm. of anhydrous sodium carbonate and 21 gm. of sodium nitrite in 200 ml. of

* *Corrected.*

water was poured slowly with stirring into 500 ml. of boiling 2.5 *N* sulphuric acid solution. The resultant solution was cooled and ammonium hydroxide was added until a brown precipitate had formed, which was filtered off. The brown precipitate was recrystallized from 95% ethanol, giving light yellow crystals. Yield, 37% of theoretical. The compound begins to decompose at 226° C.* with partial sublimation. It is insoluble in cold water, cold ethanol, acetone, benzene, and soluble in boiling water, boiling ethanol, and pyridine. Found: C, 63.40, H, 3.64, N, 7.46%. Calc. for $C_{10}H_7NO_3$: C, 63.46, H, 3.75, N, 7.40%.

Part II

5-Hydroxyquinoline-8-carboxylic Acid as an Analytical Reagent

Experimental

A Qualitative Work

An aqueous solution of the sodium salt of 5-hydroxyquinoline-8-carboxylic acid was tested both cold and after boiling, with the following ions: Al^{+++}, NH_4^+, Sb^{+++}, Ba^{++}, Be^{++}, Bi^{+++}, Cd^{++}, Ce^{++}, Cr^{+++}, Co^{++}, Cu^{++}, Au^+, Fe^{++}, Fe^{+++}, Pb^{++}, Li^+, Mg^{++}, Mn^{++}, Hg^{++}, Hg^+, Ni^{++}, Pd^{++}, Pt^{+++}, Ru^{+++}, Ag^+, Sn^{++}, Sn^{++++}, Tl^{+++}, Th^{++++}, U^{++++++}, Zn^{++}, Zr^{++}, Ir, Rh, Os. Precipitates were obtained with many of the ions. These precipitates will be investigated in the future. Only iron and ruthenium gave highly coloured solutions, ferrous iron giving a dark green solution in the cold and after heating, and ferric iron giving a brownish-green solution after boiling. Ruthenium gave a very dark bluish-black solution after boiling. The ferrous iron colour was very intense at pH about 7, even in a solution containing only 4 to 8 parts of ferrous iron in 10 million. However, this colour is very weak below pH 1.4, where the ruthenium colour was developed for the quantitative work

The colour with ruthenium does not develop in the presence of nitric acid or nitrites, and is perceptibly weakened by the addition of hydrochloric acid.

B. Quantitative Work

The standard ruthenium solution for use in establishing the curves was prepared by distilling a standard solution of ammonium chlororuthenate in dilute hydrogen bromide, using the method of Rogers, Beamish, and Russell (5). The distillate was boiled with 50 ml. of 1 : 20 hydrobromic acid and diluted to one litre. Aliquots of this solution were taken to determine the various points. The distillation was done in duplicate, the curve obtained from the two distillations being shown in Fig. 2 for a 1 cm. cell and Fig. 3 for a 5 cm. cell.

The following procedure was used. The ruthenium solution was measured with a pipette into a 150 ml. beaker. If it had not been previously boiled with dilute hydrobromic acid, it was diluted to 50 ml., 2.5 ml. of 1 : 20 hydrobromic acid (1 ml. concentrated (42%) hydrobromic acid to 20 ml. water) was added, and the solution was boiled for 10 min. It was then cooled in

* *Corrected.*

running water. If the solution had been treated previously with hydrobromic acid, this treatment was omitted.

To this solution were added 5.0 ml. of 6 *N* sulphuric acid and 10 ml. of a 0.05% by weight solution of the sodium salt of the reagent (this solution was prepared by suspending 0.5 gm. of the reagent in one litre of water, adding

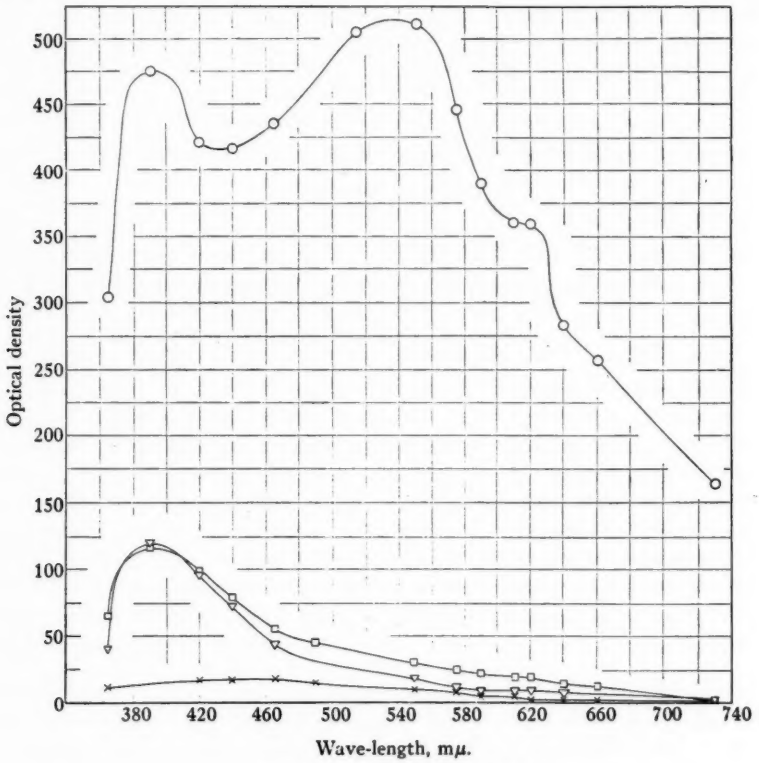

FIG. 1. *Colour absorption curve.*
○ *204 γ/50 ml. ruthenium.*
□ *400 γ/50 ml. iridium.*
▽ *400 γ/50 ml. platinum.*
× *500 γ/50 ml. osmium.*

0.5 gm. of anhydrous sodium carbonate, heating to 40° C., and allowing to cool). The solution was boiled for five minutes, cooled in running water, and diluted with water to 50 ml. in a volumetric flask. Transmission readings were taken at once.

The absorption at various wave-lengths was obtained using the above procedure, the optical density being determined with filters of various wave-lengths in a Klett–Summerson colorimeter, against a blank that had been

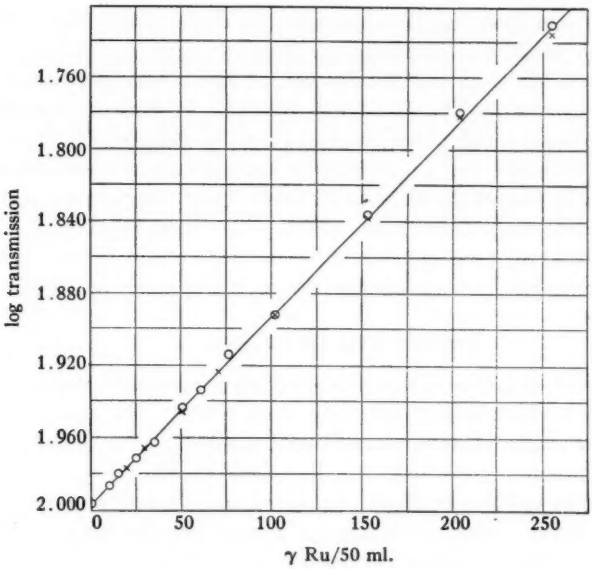

FIG. 2. *Application of Beer's law, 1 cm. cell.*

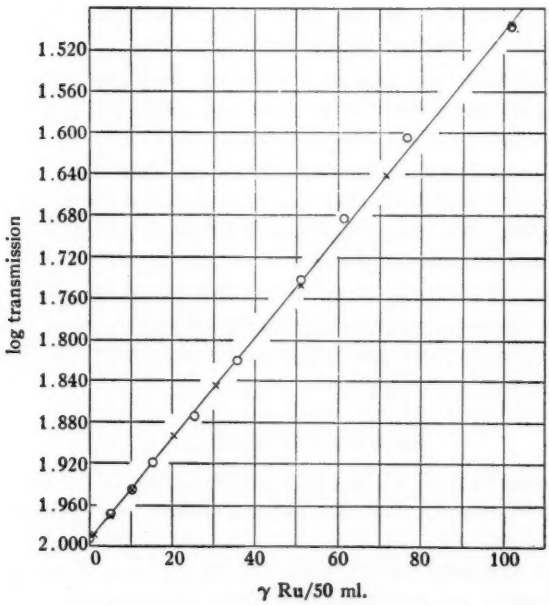

FIG. 3. *Application of Beer's law., 5 cm. cell.*

prepared in the same way but that contained no ruthenium. The curve was determined for solutions containing ruthenium, osmium, iridium, and platinum, respectively (Fig. 1). Ruthenium is difficult to separate from these metals except by distillation. The only methods at present available for determining ruthenium are based on separation by distillation. The shape of these curves indicates that for ruthenium determinations a combination of Wratten filters No. 66 and No. 15, with a Cenco–Sheard–Sanford photelometer would be suitable, and 1- or 5-cm. cells. The true blank has a slight absorption which is not constant, but changes over several hours. Hence a water blank was used, the transmission of water being taken as 100%.

Results and Discussion

The curve obtained indicates that the solutions formed with ruthenium and 5-hydroxyquinoline-8-carboxylic acid obey Beer's law. The curve does not pass through the origin because a water blank was used. The graph could be extended to higher concentrations, as it has been determined for ammonium chlororuthenate up to 750 γ in 50 ml., but this is unnecessary as aliquots may be taken to bring the amount of ruthenium present into the range 0 to 250 γ per 50 ml. The limiting perceptible concentration, using the Cenco–Sheard–Sanford photelometer with the filters as noted above, is 1 γ in 50 ml., or 1 part in 50 million. In a solution containing only the other platinum metals in small amounts and ruthenium the latter may be determined within a few per cent by this method, without a distillation (Fig. 1).

A 10 mgm. distillation was used as a standard, as Rogers, Beamish, and Russell (5) state that the method is quantitative within 1% under the most unfavourable circumstances, and is usually much better than this.

The hydroxyl group, as well as the carboxyl group, must affect the reaction, because the authors determined that quinoline-8-carboxylic acid, 5-amino-quinoline-8-carboxylic acid, and 5-hydroxyquinoline do not give any colour with ruthenium, under similar conditions.

The properties of precipitates given with 5-hydroxyquinoline-8-carboxylic acid and some of the metals will be investigated further. More derivatives of quinoline-8-carboxylic acid are being made and their effectiveness as analytical reagents will be investigated.

Acknowledgments

The authors thank Prof. F. E. Beamish of the University of Toronto, for his advice and co-operation, and Mr. R. E. Thiers, for performing the ruthenium distillations.

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PRODUCTION AND PROPERTIES OF 2,3-BUTANEDIOL

XVII. FERMENTATION OF GLUCOSE BY STRAINS OF *BACILLUS SUBTILIS*[1]

By A. C. Blackwood[2], A. C. Neish[3], W. E. Brown[3], and G. A. Ledingham[4]

Abstract

Under aerobic conditions both the Marburg and Ford types of *Bacillus subtilis* dissimilate glucose, giving carbon dioxide, acetoin, and 2,3-butanediol as the main products, and small amounts of glycerol and acetic, formic, lactic, and *n*-butyric acids. The Ford type cultures dissimilate the sugar more rapidly and also give a small amount of ethanol.

Under anaerobic conditions Marburg type cultures will not ferment glucose while the Ford type cultures dissimilate it as rapidly as they do under aerobic conditions. Some strains give 2,3-butanediol, glycerol, and carbon dioxide as the major products while others give chiefly lactic acid. As much as 86% and as little as 8% of the glucose was converted to 2,3-butanediol plus glycerol. The 2,3-butanediol was a mixture of 65% *meso*- and 35% *levo*-isomers, while the lactic acid was approximately 90% *dextro*-isomer in most cases.

It is suggested that the Ford type deserves separate species rank.

Introduction

Preliminary studies on the dissimilation of glucose by a Ford strain of *Bacillus subtilis* (7) indicated that the fermentation followed the equation:

$$3C_6H_{12}O_6 \longrightarrow 2CH_3.CHOH.CHOH.CH_3 + 2CH_2OH.CHOH.CH_2OH + 4CO_2.$$

Owing to the production of some ethanol and lactic acid, only 78% of the glucose was dissimilated according to this equation. Since these studies were all conducted with one strain of *B. subtilis* it appeared worth while to assess a number of strains to see how closely the theoretical yields could be approached.

In addition to the fermentation studies, detailed bacteriological investigations were made on a large number of *Bacillus* isolates. Most of these were isolated locally but several strains were obtained from other laboratories interested in the taxonomy of this genus. The morphological and biochemical characteristics of the Marburg and Ford type strains were compared.

Experimental

Cultures and Media

A number of *Bacillus subtilis* cultures were obtained and the fermentation characteristics were determined on the following:

B_2—A Ford strain secured from Dr. Lochhead, Central Experimental Farm, Ottawa, number 246 in his collection and used in the previous study (7).

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Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as Paper No. 45 on the Utilization of Wastes and Surpluses, and as N.R.C. No. 1469.

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[4] *Mycologist, Industrial Utilization Investigations.*

B_5—One of Ford's original cultures secured from Dr. N. R. Smith, U.S.D.A., Washington, and numbered 243 in his collection.

B_7—Secured from Dr. N. R. Smith and numbered 329 in his collection.

B_{42}—Marburg strain, number 6051 secured from the American Type Culture Collection.

B_{43}—Marburg strain secured from Dr. I. C. Gunsalus, Cornell University, Ithaca, N.Y., and numbered C_4 in his collection.

B_{44}—Ford strain secured from Dr. I. C. Gunsalus and numbered S_8 in his collection.

In addition a large number of *Bacillus* cultures were isolated and 169 were identified as *Bacillus subtilis* according to a scheme suggested by Dr. N. R. Smith (11). Carbon balances were obtained mainly under anaerobic conditions for the above named cultures and for 27 of the isolates. Only those cultures that fermented glucose rapidly were used.

In preparing the medium used, 5.0% c.p. glucose and 0.5% yeast extract (Difco) were sterilized together and, after cooling, sterile salts solution (potassium monohydrogen phosphate (0.05%), potassium dihydrogen phosphate (0.05%), magnesium sulphate heptahydrate (0.02%)) was added. A weighed amount (about 1%) of sterile calcium carbonate was added at the time of inoculation with 7.5% of a 24 hr. inoculum grown at 37° C. Preliminary experiments showed that the salts added at the above levels were sufficient. Lower levels of yeast extract gave slower fermentations.

*Analysis and Identification of Products**

Analytical methods for determining the yield of products were the same as described previously (7) except that hydrochloric acid was used to acidify the culture, and the bacteria and proteins were precipitated with zinc hydroxide according to the method of Somogyi (12).

In order that other cultures might be more easily assessed a further group was analysed only for the following products; 2,3-butanediol, glycerol, ethanol, and lactic acid. The rate of fermentation was measured daily by recording the time taken by the carbon dioxide given off to neutralize a standard amount of sodium hydroxide to the phenolphthalein end-point. When fermentation had ceased (as evidenced by a long decolorization time) the amount of residual glucose, 2,3-butanediol, glycerol, ethanol, and lactic acid was determined as follows. The solutions were cleared with zinc hydroxide. Glucose was determined by the method of Underkofler *et al.* (13) and ethanol by distillation and dichromate oxidation. The amount of lactic acid was estimated by a modification of Hillig's (2) colorimetric method. If glucose was present in more than trace amounts it was removed with copper lime treatment and the amount of 2,3-butanediol and glycerol was determined with periodate. The formic acid found on periodate oxidation and the amount of periodate reduced were determined according to Shupe's procedure

**A mimeographed bulletin has been prepared that contains full details of all the analytical methods. A copy of this is available for anyone requesting it.*

(10) except that 0.1 *M* sodium periodate was used instead of 0.01 *M* potassium periodate and excess periodate was determined by the method of Rappaport *et al.* (8).

The isolation and identification of the fermentation products were carried out by the same methods as previously described (7) except for lactic acid. The relative amounts of *dextro-* and *levo*-lactic acid were determined by preparing the silver salt of lactic benzimidazole according to the method of Moore *et al.* (6).

Results

Identification of Cultures

A detailed investigation of the morphological and biochemical characteristics of the cultures isolated in this laboratory and identified as *Bacillus subtilis* has shown that they are similar to the *subtilis* group described by Gibson (1), Knaysi and Gunsalus (3), Lamanna (4), and others. The organism is described as a motile, mesophilic, aerobic rod with a diameter less than 0.9μ. The cells from glucose nutrient agar stain evenly and are Gram-positive at 24 hr. in nutrient broth. The sporangia are not distinctly bulged and are oval to cylindrical with central to terminal thin-walled spores. The organism is V.P. positive, hydrolyzes starch, gelatin and casein and reduces nitrate to nitrite. When ammonium salts are used as the nitrogen source, acid but no gas is produced from glucose, xylose, arabinose, fructose, sucrose, maltose, glycerol, and mannitol but not from lactose. Growth on nutrient agar slopes and plates is abundant, dull, and wrinkled. Often the growth tends to adhere to the agar and, if the agar is moist, to spread rapidly.

However, considerable variation was noted among the isolates. Some difficulties were encountered in determing the characteristics of the sporangium and some isolates were first segregated because the sporangium tended to be swollen with a terminal spore. Repeated tests, however, showed that this was a variable characteristic and certainly not clearly defined. Thus, these cultures were included in the *subtilis* group. Some definite differences in colony form were noted but as reported by Gibson (1) these also were found to vary with time. The largest groups of true variants were those that did not produce diastase (16 cultures). These resemble *B. subtilis* in all other respects and were separated from the *B. pumilus* cultures, which do not produce diastase but also do not reduce nitrates to nitrites. A few cultures, contrary to type, produced some acid from lactose. Growth occurred over a wide temperature range but all fermentations were carried out at 30° to 37° C.

In the study of the rate of fermentation of glucose under mildly aerobic conditions it soon became apparent that there was a division into two rather distinct groups. The Marburg type cultures utilized the carbohydrate slowly while the Ford type cultures dissimilated the sugar quite rapidly. This difference is shown in Fig. 1. Most of the cultures were easily separated into the two groups and the rest were roughly segregated on the basis of other tests. A preliminary comparison with the results of morphological and other biochemical tests shows that this division between the Marburg and Ford

types is probably valid. Further investigation is necessary to prove the value of this proposed method of separation although both Gibson (1) and Knaysi and Gunsalus (3) use growth and production of carbon dioxide under anaerobic conditions as definite criteria.

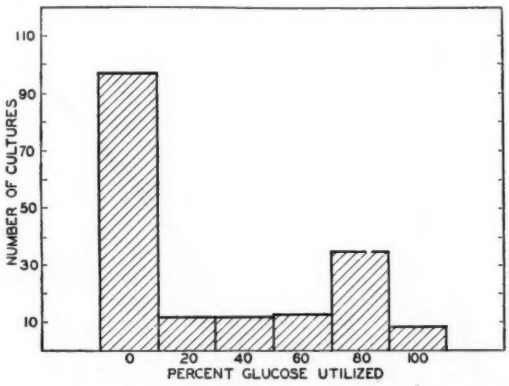

FIG. 1. *Glucose utilized by Bacillus subtilis at 37° C. in five days. The bacteria were grown aerobically on medium containing 5% glucose, 0.5% yeast extract, and 1% calcium carbonate.*

Dissimilation of Glucose by Bacillus subtilis (Marburg Type)

Although the Marburg type cultures fermented glucose very slowly it was desirable to determine the products so that a comparison might be made. The rate of carbon dioxide production by two authentic Marburg cultures, first under anaerobic (nitrogen), and then under aerobic conditions (air) is given in Fig. 2. The small initial amount of carbon dioxide given off under nitrogen may have been due to the presence of dissolved oxygen in the medium. The typical production of carbon dioxide under anaerobic conditions by a Ford culture is given for comparison. These results showed that the Marburg strains tested were strict aerobes which did not dissimilate glucose under anaerobic conditions on the media used. However, Knaysi and Gunsalus (3) found that by the addition of nitrate to the glucose medium some carbon dioxide was produced anaerobically.

Carbon balances are given in Table I for comparison of Marburg and Ford strains under aerobic conditions. Except for the fact that the Marburg cultures did not produce ethanol there was little difference.

Anaerobic Dissimilation of Glucose by Bacillus subtilis (Ford Type)

All the cultures were tested to find their rate of glucose dissimilation. Carbon balances were then determined on some of the faster fermenting strains. The product yields from three named cultures and nine isolates are given in Table II. The results are similar except for Strain 198. The best yield of 2,3-butanediol and glycerol accounts for 86% of the glucose and is somewhat better than that previously obtained. As the yield of 2,3-butanediol and

glycerol decreases, the yield of lactic acid tends to increase. With Culture 198 lactic acid is the main product; 2,3-butanediol and acetoin account for only 8.7% of the glucose, while no glycerol was found. In spite of this

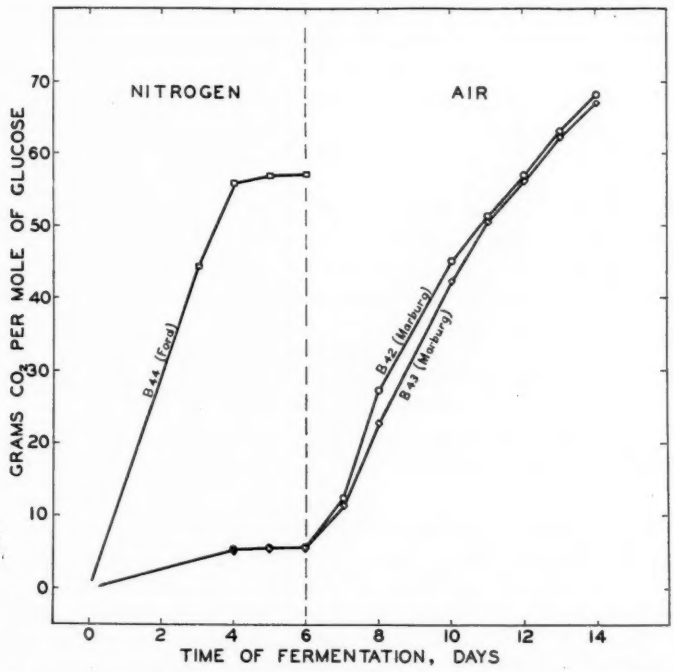

FIG. 2. *Rate of glucose dissimilation by Marburg and Ford types of Bacillus subtilis. Both types were grown anaerobically for six days and then the Marburg strains were grown aerobically for a further eight days.*

marked difference in fermentative properties it was not possible on the basis of other criteria to segregate this culture from the *subtilis* group. Possibly a larger percentage of strains producing mainly lactic acid could be found if more cultures with a slower rate of fermentation were investigated.

In order to assess the fermentation characteristics of a further group of cultures, analyses for the major products only were obtained for 18 isolates. The results are given in Table III. The product yields obtained from these cultures follow trends similar to those given in Table II. As much as 82% and as little as 8% of the glucose was converted to 2,3-butanediol and glycerol. Two cultures, like number 198, produced lactic acid as the main product.

Isolation and Identification of the Fermentation Products

Cultures 5, 7, 42, 43, 44, 106, 117, 138, 161, 198, 298, 299, and 304 were each grown in two litres of medium and the products isolated and identified by the melting point and the mixed melting point (with an authentic sample) of the

TABLE I

DISSIMILATION OF GLUCOSE UNDER AEROBIC CONDITIONS BY STRAINS OF *Bacillus subtilis*

Product	mM. product per 100 mM. glucose dissimilated		
	B42*	B43*	B2*
2,3-Butanediol	30.41	30.65	33.35
Acetoin	24.61	30.08	33.63
Glycerol	2.27	1.97	3.66
Ethanol	Nil	Nil	7.38
Formic acid	0.19	0.31	1.03
Acetic acid	4.23	3.64	4.89
n-Butyric acid	8.05	6.52	1.24
Lactic acid	1.76	1.25	1.77
Carbon assimilated	—	—	34.00
Carbon dioxide	285.0	284.0	207.58
Hydrogen	Nil	Nil	Nil
Time of fermentation, days	7	7	8
Glucose dissimilated, %	53.4	53.4	98.9
Carbon recovered, %	93.6	98.3	93.0

* *Named cultures previously described, B42 and B43, Marburg, B2, Ford strain.*

Conditions: Grown at 30° C. using 150 ml. medium in a 1000 ml. Erlenmeyer. Carbon-dioxide–free air was bubbled through at 12 to 14 ml. per min.

TABLE II

DISSIMILATION OF GLUCOSE UNDER ANAEROBIC CONDITIONS BY VARIOUS FORD TYPE STRAINS OF *Bacillus subtilis*

Product	Culture number											
	5*	7*	44*	200	161	299	298	304	107	117	138	198
	mM. product per 100 mM. of glucose dissimilated											
2,3-Butanediol	48.50	37.98	38.67	54.73	54.60	51.80	47.4	42.96	36.10	39.61	36.65	8.12
Acetoin	1.04	0.63	1.33	1.59	1.56	0.67	0.3	0.57	0.42	0.19	0	0.55
Glycerol	46.40	34.41	37.42	57.50	56.80	41.30	42.2	39.59	44.80	36.33	31.40	—
Ethanol	21.58	15.48	13.37	7.85	7.65	12.22	9.6	12.63	7.45	10.01	14.59	4.58
Formic acid	19.60	15.65	10.23	1.20	1.32	8.24	8.0	9.50	6.38	6.06	8.18	24.17
Acetic acid	0.56	1.32	1.64	—	0.16	—	—	—	—	—	—	6.99
n-Butyric acid	—	—	—	—	0.46	—	—	—	—	—	—	—
Lactic acid	24.81	54.70	47.50	23.13	17.61	38.50	44.6	54.10	62.20	59.80	73.45	137.10
Succinic acid	1.71	0.32	0.25	1.12	1.08	0.40	0.2	0.75	0.54	0.59	0.77	4.57
Malic acid	—	—	—	—	—	—	0.5	—	—	—	—	—
Carbon dioxide	112.9	85.7	93.1	114.9	117.8	102.2	97.1	88.8	77.6	87.1	72.5	4.43
Hydrogen	0	0	0	0	0.16	0.30	0.3	0	0.65	0.43	0	0
Fermentation time, days	5	6	6	8	11	9	6	11	9	11	7	11
Glucose fermented, %	99.2	99.2	99.2	99.5	99.7	99.6	99.6	99.5	90.4	99.7	99.8	52.2
Carbon recovered, %	99.5	93.2	92.0	100.0	98.0	97.8	96.4	97.1	95.7	94.0	93.7	86.4
O/R Index	1.00	1.03	1.07	0.96	0.99	0.96	1.00	0.97	0.96	1.03	0.90	1.08

* *Named cultures described previously.*

Conditions: Grown at 34° C. using 150 ml. medium in a 1000 ml. Erlenmeyer. Oxygen-free nitrogen was bubbled through at 12 to 14 ml. per min.

TABLE III

ANAEROBIC DISSIMILATION OF GLUCOSE BY VARIOUS OTHER FORD STRAINS OF *Bacillus subtilis*

Culture No.	% Glucose dissimilated	mM. product per 100 mM. glucose fermented				% of dissimilated glucose accounted for*
		2,3-Butanediol	Glycerol	Ethanol	Lactic acid	
158	98.4	55.20	54.40	13.00	16.51	97.2
205	98.8	50.95	51.80	14.50	25.61	96.9
167	99.0	50.45	51.80	12.82	32.10	98.7
165	98.4	47.60	52.50	10.18	20.65	89.3
166	98.7	50.90	48.20	19.60	32.40	101.0
164	99.3	46.90	52.20	6.71	40.00	93.4
339	96.0	55.70	42.60	20.90	17.53	96.2
345	94.5	54.60	39.40	22.42	21.45	95.7
350	97.5	56.15	34.60	21.82	28.18	98.4
211	99.0	49.40	36.41	17.52	35.09	93.9
136	77.4	40.00	33.22	13.45	49.80	88.2
108	89.3	42.55	30.85	16.81	40.95	86.9
343	98.2	38.61	32.20	19.75	41.60	85.4
346 (a)	98.8	38.40	32.15	16.92	53.51	89.7
137	99.1	47.00	21.70	21.55	47.20	92.2
172	73.6	38.10	22.20	20.80	53.38	86.3
163	53.8	7.80	Tr	4.74	134.50	77.4
210	53.5	8.01	—	3.30	142.20	80.8

** The calculation was made by assuming that from one mole of glucose is produced one mole of 2,3-butanediol, or two moles of glycerol, ethanol, or lactic acid.*

Time of fermentation was 11 days at 37° C. under anaerobic conditions (oxygen-free nitrogen), using 150 ml. medium in a 1000 ml. Erlenmeyer.

TABLE IV

A COMPARISON OF THE PROPERTIES OF 2,3-BUTANEDIOL AND LACTIC ACID PRODUCED BY VARIOUS *Bacillus subtilis* CULTURES

Culture No.	2,3-Butanediol			Lactic acid	
	$[\alpha]_D^{23}$	Refractive index at 25° C.	Boiling point, ° C.	% *dextro*	% *levo*
5	−4.86°	1.4311	177–180	87.2	12.8
44	−4.17°	1.4338	177–180	78.6	21.4
7	−3.86°	1.4332	177–180	89.2	10.8
161	−3.86°	1.4340	177–180	92.8	7.2
299	−3.89°	1.4333	177–180	93.1	6.9
298	−4.06°	1.4343	177–180	91.6	8.4
304	−4.39°	1.4345	129–134*	91.6	8.4
117	−4.63°	1.4330	177–181	95.6	4.4
138	−4.23°	1.4331	177–180	53.7	46.3
198	—	No data	—	94.6	5.4
106	−4.36°	1.4345	177–184	95.1	4.9

**At 10 cm. pressure; the others are at 76 cm.*

crystalline derivative. The Marburg cultures, numbers 42 and 43, fermented only half the glucose, and, as they produced about equal amounts of 2,3-butanediol and acetoin, it was not possible to recover sufficient of the 2,3-butanediol to determine its properties. The acetoin in this case was identified. No ethanol was obtained from these Marburg cultures, as the carbon balance data show.

Table IV gives the properties of the 2,3-butanediol and lactic acid produced by the Ford type cultures. The rotation and other properties of the 2,3-butanediol vary little among this selection of strains. However, the relative amounts of the lactic acid isomers produced vary from a slight excess of *dextro-* to nearly all *dextro*-lactic acid.

Discussion

Thirty Ford type cultures have been assessed in detail. Extreme variations in the yield of the various products have been obtained. With some strains 86% of the glucose is accounted for by the following equation:

$$3C_6H_{12}O_6 \longrightarrow 2CH_3.CHOH.CHOH.CH_3 + 2CH_2OH.CHOH.CH_2OH + 4CO_2 .$$

In the previous study (7) the yield of glycerol was somewhat lower than that theoretically possible, based on the yield of 2,3-butanediol. However, a number of cultures have now been obtained that give approximately equal molar yields of these two products. These experiments have all been carried out under the conditions previously found to give the best yield of glycerol (7). It is hoped that a further investigation of environmental conditions with individual cultures may give faster fermentation rates and possibly an increased yield of the desired products. In a previous paper (7) it was concluded that the high yields of lactic acid reported by various investigators as being produced by *Bacillus subtilis* were in part due to fermentation conditions. However, the results obtained with certain strains indicate that the culture used is also important.

The designation by the Second International Congress for Microbiology of the Marburg strain as the type strain of the *Bacillus subtilis* species has not solved the taxonomic difficulties. The investigations of Lamanna (4), Knaysi and Gunsalus (3), and Gibson (1) have shown that the strains described by Ford (5) as *subtilis* differ from the type strain sufficiently to be considered as a distinct species; in fact Gibson has separated these Ford strains and named them *Bacillus licheniformis* (Weigmann) Gibson (1). In addition to other criteria the faster rate of carbon dioxide production from glucose by the Ford type cultures has been noted. Our results show this distinction more definitely, in that under anaerobic conditions there is no fermentation by the Marburg strains and a good rate of fermentation by the Ford strains. It was possible to separate the two types on this basis alone. It is significant that in the aerobic dissimilation of glucose, although the products produced by the two types are similar, the Marburg cultures do not give ethanol. These

results are a further indication that the Ford strains of *Bacillus subtilis* are distinctive enough from the type strain (Marburg) to be given separate species rank.

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PRODUCTION AND PROPERTIES OF 2,3-BUTANEDIOL

XVIII. DISSIMILATION OF GLUCOSE BY *SERRATIA MARCESCENS*[1]

By A. C. Neish[2], A. C. Blackwood[3], F. M. Robertson[2], and G. A. Ledingham[4]

Abstract

Four strains of *Serratia marcescens* were found to dissimilate glucose under anaerobic conditions, producing 2,3-butanediol according to the equation: $C_6H_{12}O_6 \longrightarrow CH_3 . CHOH . CHOH . CH_3 + HCOOH + CO_2$. Under aerobic conditions little formic acid was produced and the reaction became $C_6H_{12}O_6 + \frac{1}{2}O_2 \longrightarrow CH_3 . CHOH . CHOH . CH_3 + H_2O + 2CO_2$. These reactions accounted for 40 to 60% of the glucose. The remainder was fermented to lactic acid (5 to 25%), ethanol (10 to 20%) and glycerol (1 to 4%). The lactic acid produced was 95 to 97% of the *levo*-isomer; the 2,3-butanediol was a *meso-dextro* mixture containing about 2% of the *dextro*-isomer.

Introduction

Pederson and Breed (7) made the first study of the products formed on dissimilation of glucose by *Serratia marcescens*. The organisms were grown on a 1.0% peptone broth containing 3% glucose and buffered with calcium carbonate. The products obtained with a typical strain were lactic acid (40%), carbon dioxide (28%), acetoin and 2,3-butanediol (18%), acetic acid (5.8%), ethanol (4.7%), and formic acid (0.2%). Some hydrogen and succinic acid were also formed, the molecular ratio of carbon dioxide to hydrogen being about 10 : 1. The lactic acid was shown to be the *levo*-isomer but the configuration of the 2,3-butanediol was not determined. Sigurdsson and Wood (8) fermented glucose solutions, containing large amounts of trimethylamine oxide, with heavily buffered, washed-cell suspensions of *S. marcescens*. The yield of lactic acid varied with the pH but was 40 to 77% for the two strains used. The other products were acetic acid, formic acid, ethanol, carbon dioxide, and acetoin. No appreciable quantities of 2,3-butanediol were reported. In some experiments more than 20% of the glucose used was unaccounted for.

Organisms that produce 2,3-butanediol, but not hydrogen, are interesting because they may give good yields of glycerol under anaerobic conditions (3, 6). Since strictly anaerobic conditions were not used by the previous workers, it was considered worth while to investigate the glucose catabolism of several strains of *S. marcescens* that were known to yield little or no hydrogen. The

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[2] *Biochemist.*

[3] *Bacteriologist.*

[4] *Mycologist.*

dissimilation of glucose was studied using four strains under both anaerobic and aerobic conditions and on a synthetic medium as well as on one containing yeast extract.

Experimental and Results

Organisms and Media

The four strains of *Serratia marcescens* used were:

S1 and S3—isolated in this laboratory and classified as *Serratia marcescens*, following the key in Bergey's Manual (2).

S2—received from the Department of National Health and Welfare, Ottawa, Canada.

S9—American Type Culture Collection Strain Number 264.

The two media used were:

(*a*) *Yeast-extract medium*—glucose (5%), yeast extract (0.5%), potassium dihydrogen phosphate (0.05%), potassium monohydrogen phosphate (0.05%), and magnesium sulphate (0.02%).

(*b*) *Synthetic medium*—composition the same as the above except that the yeast extract was replaced by 0.25% ammonium chloride.

These were both buffered with calcium carbonate (2%), which was sterilized separately and added just before use. The inoculum was incubated overnight at 37° C. in 10 ml. of a medium containing glucose (1%) and yeast extract (0.5%). This was used to inoculate 150 ml. of either of the above media.

Dissimilation of Glucose

The products of glucose dissimilation were determined under both aerobic and anaerobic conditions, using the methods previously described (3, 6).

The chief products obtained, in the absence of oxygen, were 2,3-butanediol, formic acid, ethanol, lactic acid, and carbon dioxide (Table I). The four strains gave similar results although S9 yielded considerably more succinic and lactic acids and less of the other products. All gave small amounts of acetoin and glycerol but little or no hydrogen.

The introduction of air greatly depressed formic acid production although the amounts of 2,3-butanediol, acetoin, and carbon dioxide formed were greater (Table II). Acetic acid, which may be formed by oxidation of the ethanol, appeared as a product. The fermentations were more rapid under aerobic conditions both on the synthetic medium and on that containing yeast extract. The yeast extract enabled all the strains to dissimilate nearly twice as much glucose during the first day but, in spite of this, some fermentations were completed first on the synthetic medium. The presence of yeast extract favours the production of lactic acid and depresses the formation of formic acid. These are the only effects, of addition of yeast extract, common to all strains.

TABLE I

ANAEROBIC DISSIMILATION OF GLUCOSE BY *Serratia marcescens*

Products	mM. of products per 100 mM. of glucose dissimilated			
	Strain S1	Strain S2	Strain S3	Strain S9
2,3-Butanediol	57.90	55.20	51.45	42.45
Acetoin	0.25	0.50	0.81	1.14
Glycerol	6.14	4.18	4.54	5.63
Ethanol	40.85	41.30	42.24	25.90
Lactic acid	15.70	26.50	33.09	54.15
Formic acid	48.50	44.00	39.80	27.60
Acetic acid	Nil	Nil	Nil	Nil
Succinic acid	2.98	3.34	3.41	18.80
Carbon dioxide	103.8	102.5	106.1	78.2
Hydrogen	Nil	Nil	0.52	0.27
Fermentation time, days	17	12	9	7
Glucose dissimilated, %	93.2	88.5	99.5	99.9
Carbon accounted for, %	91.4	93.0	94.6	97.5
O/R index	0.99	1.03	1.04	1.01

The bacteria were grown in the yeast-extract medium (150 ml. in a one litre Erlenmeyer flask) at 35° C. The medium was buffered with excess calcium carbonate (2%), and anaerobic conditions were maintained by continuous passage of oxygen-free nitrogen through the solution.

TABLE II

AEROBIC DISSIMILATION OF GLUCOSE BY *Serratia marcescens*

Products	mM. of products per 100 mM. of glucose dissimilated							
	Strain S1		Strain S2		Strain S3		Strain S9	
	Yeast-extract medium	Synthetic medium	Yeast-extract medium	Synthetic medium	Yeast-extract medium	Synthetic medium	Yeast-extract medium	Synthetic medium
2,3-Butanediol	62.48	58.45	56.30	59.60	55.40	52.55	41.10	48.90
Acetoin	3.31	7.45	7.51	7.52	6.56	7.92	7.10	7.59
Glycerol	1.39	1.73	1.93	1.08	1.99	1.18	1.11	Nil
Ethanol	38.30	29.80	33.25	25.30	29.65	32.80	36.10	31.10
Lactic acid	9.89	6.05	9.20	4.90	22.70	11.70	13.30	7.73
Formic acid	3.64	4.56	1.05	3.91	1.61	3.13	2.05	3.14
Acetic acid	3.43	7.78	8.40	8.40	5.52	4.94	8.19	5.55
Succinic acid	7.71	4.67	7.40	5.12	6.12	8.59	18.10	17.99
Carbon dioxide	177.8	164.8	163.3	151.5	154.5	154.0	159.0	157.2
Hydrogen	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Fermentation time, days	5	3	5	5	3	4	5	5
Glucose dissimilated, %	99.9	99.9	99.8	100.0	99.6	100.0	100.0	100.0
Carbon accounted for, %	98.8	92.0	94.4	90.0	95.4	91.4	93.1	92.4

In all experiments 150 ml. of medium was used in a one litre Erlenmeyer flask; a current of carbon-dioxide–free air was bubbled through continuously at the rate of 80 to 90 ml. per hr. The media were buffered with excess calcium carbonate (2%) and maintained at a temperature of 35° C.

Succinic acid is produced by all strains, particularly S9, which yields 12 gm. of succinic acid for every 100 gm. of glucose fermented. Its production is not influenced by aeration or by adding yeast extract to the medium.

The major products of two of these strains were isolated and identified as crystalline derivatives using the methods previously described (3, 6). The 2,3-butanediol is chiefly the *meso*-isomer but a small amount of the *dextro*-isomer is present; the lactic acid is predominantly *levo* (see Table III).

TABLE III

NATURE OF THE 2,3-BUTANEDIOL AND LACTIC ACID PRODUCED BY *Serratia marcescens*

—	Strain S1	Strain S3
Boiling point of 2,3-butanediol, ° C.	181 to 184	181 to 184
Refractive index of 2,3-butanediol, 25° C.	1.4354	1.4350
$[\alpha]_D^{25}$ of 2,3-butanediol	+0.07	+0.35
Lactic acid; per cent as *levo*-isomer	95	93
Lactic acid; per cent as *dextro*-isomer	5	7

The bacteria were grown in the yeast-extract medium buffered with calcium carbonate (2%), at 35° C. under anaerobic conditions.

Discussion

The anaerobic dissimilation of glucose by *Serratia marcescens* has not been studied previously. Pederson and Breed's (7) studies on mildly aerobic glucose dissimilation by this species agree in a general way with our results, except that we obtained little or no hydrogen. The other differences may be due to differences in the media, between strains, or in the degree of aeration.

Succinic acid is produced by all strains, particularly S9. Since it is produced just as readily on a medium containing glucose as the sole source of carbon as it is on a medium containing added yeast extract, it is regarded as a product originating from glucose rather than from proteins.

Bacteria are able to convert glucose to 2,3-butanediol by four different fermentations. The best known of these is described by the equation:

$$C_6H_{12}O_6 \longrightarrow CH_3 . CHOH . CHOH . CH_3 + H_2 + 2CO_2 .$$

This reaction is catalyzed by *Aerobacter aerogenes* (4, 5) *Aerobacillus polymyxa* (1) and *Aeromonas hydrophila* (9), and has been known for almost 40 years. Recently it has been found (3, 6) that the Ford type of *Bacillus subtilis* produces 2,3-butanediol according to the following equation:

$$3C_6H_{12}O_6 \longrightarrow 2CH_3 . CHOH . CHOH . CH_3 + 2CH_2OH . CHOH . CH_2OH + 4CO_2 .$$

The other two fermentations are carried out with *Serratia marcescens:*

$$C_6H_{12}O_6 \longrightarrow CH_3 . CHOH . CHOH . CH_3 + HCOOH + CO_2$$

$$C_6H_{12}O_6 + \tfrac{1}{2} O_2 \longrightarrow CH_3 . CHOH . CHOH . CH_3 + H_2O + 2CO_2 .$$

The first of these describes the fermentation of glucose under anaerobic conditions, the second its dissimilation under aerobic conditions.

All these bacteria catalyze parallel reactions, particularly the lactic acid fermentation, or the ethanol fermentation. In addition, *S. marcescens* produces some of its 2,3-butanediol by the diol–glycerol fermentation, and *B. subtilis* a little by the diol–formic-acid fermentation. The closest approach to a homofermentative organism, as far as these equations are concerned, is found with certain strains of *B. subtilis* which dissimilated 86% of the glucose according to the above equation for the 2,3-butanediol–glycerol fermentation, and strains of *A. aerogenes* which give 90% of the 2,3-butanediol–hydrogen fermentation.

The equations given above do not explain the mechanism of the formation of 2,3-butanediol from sugars but merely state the over-all yields of products that can be expected. It is possible that organisms will be found that dissimilate practically all the glucose according to one or other of these equations.

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PRODUCTION AND PROPERTIES OF 2,3-BUTANEDIOL

XIX. CYCLIC ACETALS AND KETALS DERIVED FROM *LEVO*-2,3-BUTANEDIOL[1]

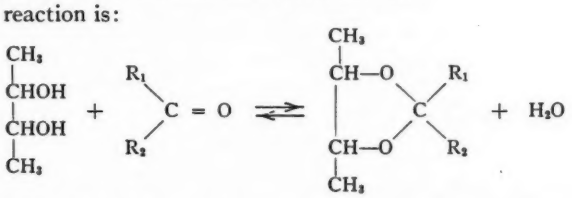
By A. C. Neish[2] and F. J. Macdonald[2]

Abstract

Optically active 4,5-dimethyl-1,3-dioxacyclopentane derivatives can be easily obtained by condensing *levo*-2,3-butanediol with aldehydes or ketones in the presence of small amounts of strong acids at ordinary temperatures. Condensations were effected with formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, isobutyraldehyde, 2-ethylbutyraldehyde, 2-ethylhexaldehyde, benzaldehyde, furfural, acrolein, acetone, methyl ethyl ketone, methyl isobutyl ketone, methyl amyl ketone, diethyl ketone, acetophenone, cyclohexanone, acetoin, and diacetyl. The expected products were obtained, two compounds being formed from diacetyl since one or both carbonyl groups may react. Two compounds were obtained from formaldehyde also; in addition to the expected product (*levo*-4,5-dimethyl-1,3-dioxacyclopentane), 15 to 20% of *levo*-6,7-dimethyl-1,3,5-trioxacycloheptane was obtained.

Backer (1) observed that 2,3-butanediol condensed readily with formaldehyde, acetaldehyde, acetone, methyl ethyl ketone, cyclohexanone, benzaldehyde, and ethyl cyclohexanone-4-1-carboxylate to give 4,5-dimethyl-1,3-dioxacyclopentane derivatives. The sample of diol he used was prepared by fermentation and was probably the *meso-dextro* mixture produced by *Aerobacter aerogenes*, which is 90 to 95% of the *meso*-isomer. If this were the case Backer's compounds were *meso*-4,5-dimethyl-1,3-dioxacyclopentane derivatives. The present work is concerned with the optically active *levo*-4,5-dimethyl-1,3-dioxacyclopentane derivatives prepared from the pure *levo*-2,3-butanediol produced by *Aerobacillus polymyxa* (2).

The general reaction is:



A series of aldehydes and ketones were found to give the expected reaction products. The compounds prepared are listed in Table I, together with their physical properties. The molar refractions agree well with the calculated values. This, in conjunction with the analytical data given in the experimental section, is taken as proof of their structure. In general the *levo*-isomers have a lower boiling point, refractive index and density than the

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TABLE I

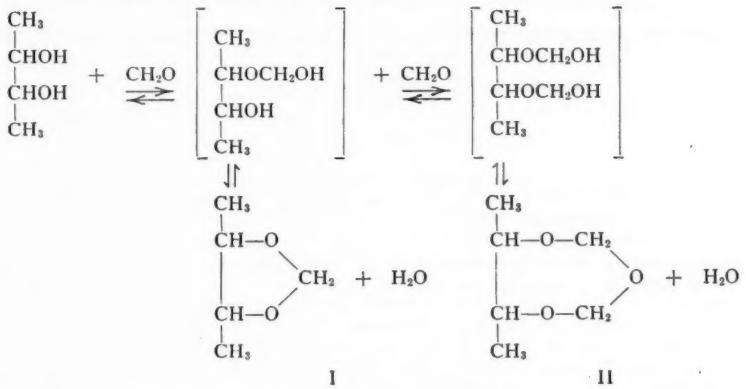
PHYSICAL PROPERTIES OF CYCLIC ACETALS AND KETALS DERIVED FROM *levo*-2,3-BUTANEDIOL

Compound	Boiling point, °C. at 760 mm.	Refractive index at 25° C.	Density d_4^{25}	Optical rotation $[\alpha]_D^{25}$ (degrees)	Molar refraction	
					Calculated	Found
levo-4,5-Dimethyl-1,3-dioxacyclopentane	97	1.3960	0.9346	− 24.9	26.38	26.25
levo-2,4,5-Trimethyl-1,3-dioxacyclopentane	103	1.3924	0.8914	− 21.8	30.98	31.02
levo-4,5-Dimethyl-2-ethyl-1,3-dioxacyclopentane	127	1.4014	0.8868	− 14.0	35.59	35.58
levo-4,5-Dimethyl-2-propyl-1,3-dioxacyclopentane	150	1.4075	0.8824	− 14.2	40.19	40.17
levo-4,5-Dimethyl-2-isopropyl-1,3-dioxacyclopentane	140	1.4062	0.8822	− 16.5	40.19	40.16
levo-4,5-Dimethyl-2-(2-ethylbutyl)-1,3-dioxacyclopentane	180	1.4202	0.8842	− 16.9	49.42	49.56
levo-4,5-Dimethyl-2-(2-ethylhexyl)-1,3-dioxacyclopentane	215	1.4275	0.8874	− 14.9	58.62	58.69
levo-4,5-Dimethyl-2-phenyl-1,3-dioxacyclopentane	230	1.4982	1.0274	− 28.1	50.71	50.76
levo-4,5-Dimethyl-2-vinyl-1,3-dioxacyclopentane	132	1.4173	0.9155	− 24.3	35.19	35.21
levo-4,5-Dimethyl-2-furyl-1,3-dioxacyclopentane	206	1.4645	1.0770	− 26.6	43.58	43.12
levo-2,2,4,5-Tetramethyl-1,3-dioxacyclopentane	110	1.3914	0.8669	− 22.1	35.59	35.71
levo-2,4,5-Trimethyl-2-ethyl-1,3-dioxacyclopentane	134	1.4025	0.8749	− 18.8	40.19	40.18
levo-2,4,5-Trimethyl-2-isobutyl-1,3-dioxacyclopentane	166	1.4110	0.8621	− 14.0	49.94	49.61
levo-2,4,5-Trimethyl-2-amyl-1,3-dioxacyclopentane	192	1.4178	0.8652	− 17.0	54.00	54.09
levo-2,4,5-Trimethyl-2-phenyl-1,3-dioxacyclopentane	219	1.4868	0.9989	− 40.7	55.31	55.12
levo-2,2-Diethyl-4,5-dimethyl-1,3-dioxacyclopentane	155	1.4109	0.8808	− 12.6	44.79	44.61
levo-2,4,5-Trimethyl-2-(*dl*-α-hydroxy ethyl)-1,3-dioxacyclopentane	181	1.4273	0.9901	− 21.0	41.71	41.56
dexiro-2,4,5-Trimethyl-2-aceto-1,3-dioxacyclopentane	177	1.4170	0.9857	+ 13.15	40.37	40.36
levo-Bis-2,2′-(2,4,5-trimethyl-1,3-dioxacyclopentane)	222	1.4450	1.0281	−101.5	59.87	59.61
levo-6,7-Dimethyl-1,3,5-trioxacycloheptane	152	1.4299	1.0336	− 61.2	32.67	33.01
levo-3,4-Dimethyl-2,5-dioxa-1-spirodecane	190	1.4438	0.9546	− 7.0	47.29	47.35

analogous compounds prepared by Backer (1). For example, *levo*-2,2,4,5-tetramethyl-1,3-dioxacyclopentane boils at 110° C. while Backer's analog has a boiling point of 117.5° to 118.5° C.

Two compounds were obtained on condensing formaldehyde with *levo*-2,3-butanediol. If the formaldehyde is bubbled into a mixture of the diol and hydrochloric acid, and the temperature allowed to rise to the point of reflux, the normal reaction product, *levo*-4,5-dimethyl-1,3-dioxacyclopentane (I), is the only one obtained. If, however, excess formaldehyde is bubbled into the *levo*-2,3-butanediol containing a small amount of hydrochloric acid and the mixture is kept cool until addition of the formaldehyde is completed, then *levo*-6,7-dimethyl-1,3,5-trioxacycloheptane (II) may be obtained in yields up to 20% of the theoretical.

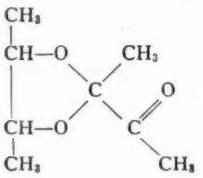
The formation of these compounds may be visualized as follows:



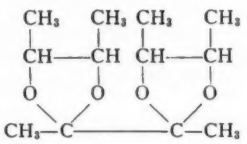
I II

The intermediate hemiacetals were not isolated in the pure state but it was possible to demonstrate that intermediates with high boiling points were present in the reaction mixture.

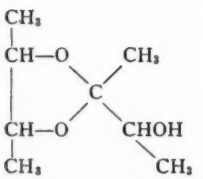
Two compounds were obtained from diacetyl as expected. These were *dextro*-2,4,5-trimethyl-2-aceto-1,3-dioxacyclopentane (III) and *levo*-bis-2,2′-(2,4,5-trimethyl-1,3-dioxacyclopentane) (IV).



III



IV



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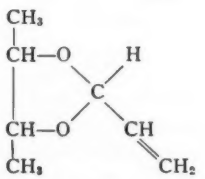
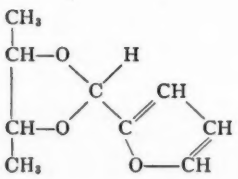
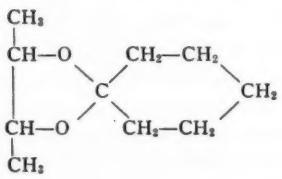


VI



VII



VIII

Acetoin condenses readily to give the hydroxyketal V, which is the only compound of those prepared that is miscible with water; it is also miscible with Skelly-solve. Acrolein condenses smoothly to give the vinylacetal VI in yields about 60% of the theoretical. Furfural reacts to give the furylacetal VII and cyclohexanone to give *levo*-3,4-dimethyl-2,5-dioxa-1-spirodecane (VIII.) The *meso*-isomer of the latter compound was prepared by Backer (1).

These compounds are resistant to alkaline hydrolysis but are readily hydrolysed by dilute acids to the compounds from which they were made. Like ethers they form peroxides when exposed to air, particularly in the light.

l-2,3-Butanediol reacts with ketones very readily; it reacts more rapidly than 1,3-butanediol, 1,3-propanediol, 1,2-propanediol, or ethanediol.

Experimental

General

The reactions were usually carried out by mixing *levo*-2,3-butanediol and the carbonyl compound in equimolar amounts and adding 1 to 5 ml. of concentrated sulphuric or hydrochloric acid per mole. This mixture was shaken or stirred and the temperature allowed to rise to 40° to 50° C. In most cases the products were insoluble in water and a two phase system formed. After the reaction was judged to be complete, the water layer was separated, and discarded, the acetal layer was washed with saturated sodium bicarbonate solution, filtered through anhydrous sodium sulphate and fractionally distilled through a column filled with glass helices or Stedman packing. The product was refractionated, and measurements made of its density, refractive index, optical rotation, and boiling point. A sample was analysed as a check on its identity. This was done by hydrolysing an accurately weighed amount of the compound with normal hydrochloric or sulphuric acid in a pressure bottle (one hour at 100° C.) and determining the 2,3-butanediol in the hydrolysate. The diol was determined by treating a suitable aliquot with 5 ml. of 0.1 *M* sodium periodate for one hour at room temperature; the periodate reduced was then determined iodometrically by the procedure of Rappaport, Reifer, and Weinmann (4). This procedure worked well on all the compounds except those derived from formaldehyde and diacetyl. These were difficult to hydrolyse quantitatively, particularly the latter. They were analysed by combustion.

Because of the reversible nature of the condensation the yields obtained are minimum values. There is no evidence of side reactions; the yields are not quantitative because the reaction reaches an equilibrium. It is not always certain that enough time was allowed for the equilibrium value to be reached.

levo-4,5-Dimethyl-1,3-dioxacyclopentane and levo-6,7-Dimethyl-1,3,5-trioxacycloheptane

Five moles of *levo*-2,3-butanediol and 5 ml. of concentrated hydrochloric acid were mixed in a two litre, three-necked flask equipped with a stirrer and cooled in a water-bath. Paraformaldehyde (8 moles) was heated in a separate

flask and the gaseous formaldehyde thus obtained bubbled into the acid–diol mixture, which warmed spontaneously to 50° to 60° C. After addition of the formaldehyde was complete (1.5 hr.) 20 ml. of hydrochloric acid was added and the solution warmed gently until it became diphasic. The top layer was separated, washed with saturated sodium bicarbonate solution, filtered through anhydrous sodium sulphate, and distilled through a 2 ft. column packed with glass helices. The products obtained were (*a*) a diphasic azeotrope boiling at 81° to 82° C.; (*b*) a liquid boiling at 97° C., and (*c*) a liquid boiling at 152° C.

The azeotrope had a top layer, the volume of which was 7.0 times that of the bottom. This top layer was separated and on distillation gave some more azeotrope, b.p. 81° to 82° C., but chiefly the liquid, b.p. 97° C. This compound is *levo*-4,5-dimethyl-1,3-dioxacyclopentane and the azeotrope is that formed with water. Its physical properties are given in Table I. It is a colourless liquid with an agreeable odour. Yield, 63% of theory, based on the diol. Found: C, 58.9, 58.9; H, 9.98, 9.80%. Cal. for $C_5H_{10}O_2$: C, 58.8; H, 9.87%. The *meso*-isomer of this compound (b.p. 102° to 103° C.) was prepared by Backer (1).

The liquid with b.p. 152° C. was redistilled and analysed. Found: C, 54.5, 54.5; H, 9.18, 9.12%; halogen, nil. Calc. for $C_6H_{12}O_3$: C, 54.5; H, 9.15%. It is a colourless liquid, immiscible with water, having an odour similar to that of the formal. On hydrolysis with acid it gives two moles of formaldehyde per mole of 2,3-butanediol. The only logical structure to assign to this compound on the basis of these facts is *levo*-6,7-dimethyl-1,3,5-trioxacycloheptane. Its physical properties are given in Table I. The yield was 14% of theory, based on the diol.

Backer (1) did not obtain a compound analogous to this. If the condensation is allowed to proceed rapidly by adding all of the acid at the start and letting the mixture warm up, it is not obtained in any appreciable amounts. Its yield can be increased to about 20% by carrying out the condensation at lower temperatures. An attempt was made to isolate intermediate hemiacetals by neutralizing the solution with sodium hydroxide after the formaldehyde (8 moles) had been bubbled into the mixture of diol (5 moles) and hydrochloric acid (5 ml.). The monophasic reaction mixture was then distilled, to give the formal–water azeotrope (0.6 moles), formaldehyde, unchanged *levo*-2,3-butanediol (2 moles) and 230 ml. of a viscous, colourless liquid boiling over the range 103° to 225° C. (10 mm.). This fraction had an observed optical rotation (*D* line) of $-59.6°$ per decimetre. After it had stood overnight at room temperature it was redistilled and 113 ml. of the formal–water azeotrope and 85 ml. of *levo*-2,3-butanediol obtained. Apparently there are high-boiling unstable intermediates formed, but their constitution is uncertain since they could not be obtained in a pure condition owing to their instability.

levo-2,4,5-Trimethyl-1,3-dioxacyclopentane

Paraldehyde (6 moles) and *levo*-2,3-butanediol (6 moles) were mixed and treated with concentrated sulphuric acid (15 gm.). After reaction had continued for one hour at 40° to 50° C. the top layer was separated and the bottom layer extracted with ether. The extract was combined with the acetal layer, washed with sodium bicarbonate solution, filtered through anhydrous sodium sulphate and distilled through a Stedman column. The acetal was obtained as a colourless liquid, b.p. 103° C., with a very pleasant odour (yield, 34% of theory). It was refractionated and its physical properties determined (see Table I). Equivalent weight (periodate reduction): calc., 58.1; found: 58.0. Backer (1) prepared the *meso*-isomer of this compound (b.p. 108° to 109° C.).

An attempt was made to prepare a 1,3,5-trioxacycloheptane derivative by mixing acetaldehyde (11.4 moles) with *levo*-2,3-butanediol (3.8 moles) and adding a trace of hydrochloric acid. The acetal and paraldehyde were the only products obtained.

levo-4,5-Dimethyl-2-ethyl-1,3-dioxacyclopentane

Propionaldehyde (5 moles) and *levo*-2,3-butanediol (5 moles) were mixed and treated with hydrochloric acid (5 ml.) added with shaking (15 min.). The mixture was kept cooled below 50° C. The top layer was then separated, treated as usual, and fractionated through a Stedman column. The product is a colourless liquid, b.p. 126° to 127° C., with an odour similar to that of the acetal (yield, 86% of theory). It was refractionated through a column packed with glass helices and its physical properties determined (Table I). Equivalent weight (periodate reduction): calc., 65.1; found, 65.6.

levo-4,5-Dimethyl-2-propyl-1,3-dioxacyclopentane

This was prepared from *n*-butyraldehyde and *levo*-2,3-butanediol by the general procedure using hydrochloric acid. The product is a liquid, b.p. 149° to 150° C. (yield, 75% of theory). It was refractionated and its physical properties determined (Table I). Equivalent weight (periodate reduction): calc., 72.1; found, 72.0.

levo-4,5-Dimethyl-2-isopropyl-1,3-dioxacyclopentane

This was prepared from isobutyraldehyde and *levo*-2,3-butanediol by the general procedure using hydrochloric acid. The product is a colourless liquid, b.p. 139° to 140° C. (yield, 58% of theory). It was refractionated and its physical properties determined (Table I). Equivalent weight (periodate reduction): calc., 72.1; found, 71.6.

levo-4,5-Dimethyl-2-(2-ethylbutyl)-1,3-dioxacyclopentane

This was prepared from 2-ethylbutyraldehyde and *levo*-2,3-butanediol by the general procedure using hydrochloric acid. This acetal is a colourless liquid, b.p. 124° to 125° C. (134 mm.); yield, 77% of theory. It was refractionated to give a liquid, b.p. 135° C. (200 mm.). Its physical properties are shown in Table I. Equivalent weight (periodate reduction): calc., 86.1; found, 86.5.

levo-4,5-Dimethyl-2-(2-ethylhexyl)-1,3-dioxacyclopentane

This was prepared from 2-ethylhexaldehyde and *levo*-2,3-butanediol, by the general procedure, using hydrochloric acid. It is a colourless liquid, b.p. 147° to 148° C. (100 mm.); yield, 66% of theory. It was refractionated and its physical properties determined (Table I). It was necessary to use some methanol to bring it into solution for determination of its equivalent weight (periodate reduction): calc., 100.2; found, 100.7.

levo-4,5-Dimethyl-2-phenyl-1,3-dioxacyclopentane

This was prepared from benzaldehyde and *levo*-2,3-butanediol, by the general procedure, using hydrochloric acid. An emulsion formed which was treated with solid sodium bicarbonate and enough anhydrous sodium sulphate to remove the water. The filtrate was fractionated to give a colourless liquid, b.p. 153° to 154° C. (75 mm.); yield, 75% of theory. It was refractionated and its physical properties determined (Table I). Equivalent weight (periodate reduction): calc., 89.1; found, 89.8. The *meso*-isomer of this compound was prepared by Backer (1).

levo-4,5-Dimethyl-2-vinyl-1,3-dioxacyclopentane

This was prepared from freshly distilled acrolein (0.8 moles) and *levo*-2,3-butanediol (0.8 moles) by treating with hydrochloric acid (1 ml.) and shaking for 15 min. The top layer was purified and distilled as usual. The acetal was a colourless liquid boiling at 131° to 132° C. (yield, 60% of theory). It was refractionated and its physical properties determined (Table I). Equivalent weight (periodate reduction): calc. 64.1; found, 63.9. About 0.2 mole of acrolein was recovered unchanged. The yield in this reaction is high compared to that in the reaction between ethanol and acrolein.

levo-4,5-Dimethyl-2-furyl-1,3-dioxacyclopentane

Furfural and *levo*-2,3-butanediol were mixed and treated with hydrochloric acid. It is important not to use sulphuric acid, which causes tar formation. After reaction had continued for two hours the lower layer (acetal) was separated and the top layer extracted with ether, the extract being added to the acetal layer. After washing with sodium bisulphite and sodium bicarbonate solutions it was dried with sodium sulphate and distilled through the Stedman column. This acetal is a colourless liquid, b.p. 140° C. (100 mm.); yield, 50% of theory. Its odour is something like that of furfural. It was refractionated; its physical properties are given in Table I. Equivalent weight (periodate reduction): calc., 84.1; found, 84.1.

levo-2,2,4,5-Tetramethyl-1,3-dioxacyclopentane

This was prepared from acetone and *levo*-2,3-butanediol, using 3 ml. of hydrochloric acid per mole of diol. The reaction was run 14 hr. with stirring. This is longer than is necessary. The top layer was treated as usual and distilled through a column filled with glass helices. This ketal is a liquid, b.p. 110° C., with a camphor-like odour (yield, 52% of theory). Equivalent weight (periodate reduction): calc., 65.1; found, 65.0. Backer prepared the

meso-isomer of this compound, b.p. 118° C. The *levo*-isomer gives a diphasic azeotrope with water, b.p. 86° C., the volume of the ketal layer being 6.7 times that of the water layer.

levo-2,4,5-Trimethyl-2-ethyl-1,3-dioxacyclopentane

This was prepared from methyl ethyl ketone (5 moles) and *levo*-2,3-butanediol, using 25 ml. of hydrochloric acid. The mixture was stirred 2½ hr. and the product worked up as usual. The ketal was obtained as a colourless liquid, b.p. 133° to 134° C., with an odour very much like that of camphor (yield, 60% of theory). It was refractionated through a column packed with glass helices and its physical properties determined (Table I). This compound has been described (3) but its properties are given again since the values published previously are slightly in error. Backer (1) prepared the *meso*-isomer of this compound, b.p. 141° to 143° C. The *levo*-isomer gives a diphasic azeotrope with water, b.p. 91° C.; the volume of the ketal layer is 3.0 times that of the water.

levo-2,4,5-Trimethyl-2-isobutyl-1,3-dioxacyclopentane

This was prepared from methyl isobutyl ketone and *levo*-2,3-butanediol using 5 ml. of hydrochloric acid per mole of diol. After 2½ hr. the top layer was separated, washed with sodium bicarbonate and distilled with water to separate it from 2,3-butanediol. The ketal–water azeotrope distils at 97° C.; it forms two layers, the volume of the ketal layer being 1.25 times that of the water layer. The ketal was dried with sodium sulphate and refractionated, this gave a colourless liquid, b.p. 120° to 121° C. (200 mm.). Its physical properties are given in Table I (yield, 43% of theory). Equivalent weight (periodate reduction): calc., 86.1; found, 86.3.

levo-2,4,5-Trimethyl-2-amyl-1,3-dioxacyclopentane

This was prepared from *levo*-2,3-butanediol and methyl *n*-amyl ketone using the same procedure as for the isobutyl ketal. The ketal–water azeotrope boiled at 98.5° to 99° C.; the volume of the ketal layer was 0.57 times that of the water. The dry ketal was a colourless liquid, b.p. 143° to 144° C. (200 mm.). The physical properties are listed in Table I (yield, 38% of theory).

levo-2,4,5-Trimethyl-2-phenyl-1,3-dioxacyclopentane

Acetophenone (5 moles) and *levo*-2,3-butanediol (5 moles) were mixed with 25 ml. of hydrochloric acid and warmed to 80° C. for one hour. The mixture was then stirred overnight at room temperature. The ketal was obtained as a colourless liquid; b.p. 146° to 147° C. (93 mm.); yield, 44% of theory. It was refractionated and its physical constants measured (Table I). Its odour is suggestive of violets. Equivalent weight (periodate reduction): calc., 96.1; found, 96.7.

levo-4,5-Dimethyl-2,2-diethyl-1,3-dioxacyclopentane

This was prepared from *levo*-2,3-butanediol (1 mole) and diethyl ketone (1 mole) by treating with 5 ml. of hydrochloric acid and heating to 60° to

70° C. for 30 min. with stirring. This ketal was obtained as a liquid, b.p. 154° to 155° C. (yield, 50% of theory). It was refractionated and its physical constants determined. Equivalent weight (periodate reduction): calc., 79.1; found, 79.7.

levo-3,4-Dimethyl-2,5-dioxa-1-spirodecane

This was prepared from cyclohexanone and *levo*-2,3-butanediol by the general procedure. This ketal is a colourless liquid, b.p. 142° to 143° C. (200 mm.); yield, 78% of theory. It was refractionated through a column packed with glass helices and its physical constants measured (Table I). Equivalent weight (periodate reduction): calc., 85.1; found, 84.9. The *meso*-isomer of this compound was prepared by Backer (1).

levo-2,4,5-Trimethyl-2-(dl-α-hydroxyethyl)-1,3-dioxacyclopentane

Three moles of *levo*-2,3-butanediol were mixed with 3 moles of *dl*-acetoin and sulphuric acid (17 ml.) added dropwise with shaking. The reaction mixture was allowed to stand for four days at room temperature. The product was extracted with ether, some water being added during the extraction to cause formation of two layers. The ketal was obtained as a liquid, b.p. 129° C. (143 mm.); yield, 38% of theory. Its physical properties are listed in Table I. Equivalent weight (periodate reduction): calc., 40.0; found, 40.1. Since this ketal gives a mole of acetoin on hydrolysis it is possible to determine its equivalent weight by measuring the acetic acid formed on oxidation of the hydrolysate with periodic acid. Equivalent weight (acid formed on oxidation with periodate): calc., 160.2; found, 160.8.

dextro-2,4,5-Trimethyl-2-aceto-1,3-dioxacyclopentane and levo-Bis-2,2'-(2,4,5-trimethyl-1,3-dioxacyclopentane)

Diacetyl (3 moles) and *levo*-2,3-butanediol (6 moles) were mixed and 1 ml. of hydrochloric acid added. The mixture was stirred continuously. It heated spontaneously to 50° C. but after one hour two phases had failed to develop so 50 ml. of hydrochloric acid was added. After standing 14 hr. a further 44 ml. of hydrochloric acid was added, the solution warmed to 50° C., and stirred; after 30 min. it became cloudy. The stirring was continued for five hours more. The top layer was then separated and combined with an ether extract of the bottom layer. After washing in the usual manner this was distilled through a two-foot column packed with glass helices. Two new compounds were obtained (*a*) a pale-yellow liquid boiling at 174° C. and (*b*) a colourless liquid boiling at 169° to 171° C. (200 mm.).

The liquid, b.p. 174° C., was washed with water to remove any unchanged *levo*-2,3-butanediol, and redistilled to give an almost colourless liquid, b.p. 134° to 135° C. (200 mm.). (Yield, 13% of theory.) This is the keto–ketal III. Its physical properties are listed in Table I. It forms a hydrazone immediately when treated with 2,4-dinitrophenylhydrazine dissolved in dilute hydrochloric acid whereas all the other ketals only form one after warming (hydrolysis). Found: C, 60.7, 60.5; H, 9.07, 8.97%. Calc. for $C_8H_{14}O_3$: C, 50.7; H, 8.91%. The higher boiling ketal was refractionated to give a

colourless liquid, b.p. 170° to 171° C. (200 mm.); yield, 24% of theory. This compound is *levo*-bis-2,2′-(2,4,5-trimethyl-1,3-dioxacyclopentane) (IV). Its physical properties are listed in Table I. Found: C, 62.5, 62.4; H, 9.54, 9.63%. Calc. for $C_{12}H_{22}O_4$: C, 62.6; H, 9.63%.

When an equimolecular mixture of diacetyl and *levo*-2,3-butanediol is treated with a little sulphuric acid a white, crystalline compound is obtained in addition to the liquids described above (yield, about 8%). These crystals dissolve in methanol to give a colourless solution, which turns yellow on boiling. They have an indefinite melting point (65° to 72° C.), which depends on the rate of heating. The melt is yellow but on solidification it again becomes white. On boiling with very dilute sulphuric acid, diacetyl and 2,3-butanediol were obtained in equimolecular yields. This compound may be a monohemiacetal or the combination may be analogous to that of water of crystallization.

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THE CATALYTIC ACTION OF ALUMINIUM SILICATES

I. THE DEHYDRATION OF BUTANEDIOL-2,3 AND BUTANONE-2 OVER ACTIVATED MORDEN BENTONITE[1]

By A. N. Bourns[2] and R. V. V. Nicholls[3]

Abstract

The dehydration of butanediol-2,3 over activated Morden bentonite has been investigated. Below 350° C., liquid products butanone-2, isobutyraldehyde, and the isobutyral of butanediol-2,3 were produced. Butanone-2 was obtained in 86% yield. At higher temperatures, 450° to 700° C., gaseous decomposition occurred. Only small amounts of butadiene were formed from the dry glycol while a 25% diolefin yield resulted using water vapour as diluent. The decomposition of dry butanone-2 over bentonite gave only traces of butadiene. Yields of diolefin increased using water as diluent, 44.8% being obtained at 700° C. with a ketone–water molar ratio of 1 to 40. Butanone-2 was considered intermediate in the formation of butadiene from butanediol-2,3.

Introduction

The fall of the Malay Peninsula, resulting in the loss of almost our entire source of natural rubber, initiated an intensive investigation of all possible methods for the production of butadiene. One of the first methods considered was the dehydration of butanediol-2,3. In Canada, research on the production of *levo*-butanediol-2,3 by the fermentation of wheat mashes by *Aerobacillus polymyxa* was conducted by the Division of Applied Biology of the National Research Council, Ottawa (11). The results of this work indicated that the glycol could be obtained in yields sufficient to warrant a consideration of its use as an industrial chemical.

The investigation reported in this paper deals with a study of the dehydration of butanediol-2,3 in the vapour phase over activated Morden bentonite. Morden bentonite is a Canadian clay mined at Morden, Manitoba, and can be obtained in considerable quantities at a relatively low cost. Previous work in these laboratories had shown that this clay, when activated by treatment with acid, yields an extremely active dehydration catalyst (17). The present investigation was carried out at both low temperatures, giving liquid mono-dehydration products, and at high temperatures, resulting in a decomposition into gaseous products. From preliminary experiments, it appeared likely that the results of the high temperature decomposition might be accounted for by a decomposition of the products of the low-temperature dehydration. Consequently, the investigation was extended to a study of the decomposition of the main low-temperature product, butanone-2, over the bentonite catalyst.

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Previous to the present synthetic rubber program, studies on butanediol-2,3 have been restricted, for the most part, to its mono-dehydration to butanone-2. Langedijk (10), in a review article on butanone-2 in 1938, listed the dehydration of butanediol-2,3 as one of the methods for the production of this ketone. Details of the process were not given, however. Recently, a process has been patented (6) for the liquid-phase dehydration of glycols to aldehydes and ketones, and the dehydration of butanediol-2,3 is given as an example. Akabori (1) investigated the liquid-phase dehydration in the presence of 25% sulphuric acid and obtained isobutyraldehyde in addition to butanone-2. Denivelle (4) carried out the vapour-phase dehydration of the glycol over a variety of catalysts, including sulphuric acid, phosphoric acid, phosphoric anhydride, basic oxides, and zinc chloride, in an attempt to produce butadiene. In all cases, butanone-2 only was obtained. Gutner and Tishchenko (7) passed butanediol-2,3 in the presence of steam over magnesium chloride at 350° C. and obtained a 20% yield of butanone-2 and a 2.9% yield of butadiene. In the absence of steam, the yield of the ketone was 26.2% and of butadiene 4%. Using magnesium sulphate at 300° to 320° C., these workers obtained a 40.6% yield of butanone-2 as well as a 2.6% yield of butadiene.

At the beginning of the present synthetic rubber program, efforts to obtain butadiene directly from butanediol-2,3 were renewed. This investigation was conducted in the United States by the Northern Regional Research Laboratory of the U.S. Department of Agriculture. A report from this laboratory (14) disclosed that 70 catalysts in all had been studied for the direct dehydration of the glycol. One-pass yields of butadiene seldom reached more than 20%, the main product being butanone-2, with methyl vinyl carbinol and butylene oxide as possible by-products. Winfield (18) recently has reported single pass conversions of 60% to butadiene and 80% to butadiene plus butanone-2 by passing the glycol under reduced pressure at 350° C. over thorium oxide.

Very little has been reported on the vapour-phase decomposition of butanone-2 over dehydration catalysts. Mitchell and Reid (12) studied the decomposition of this ketone over silica gel at 500° C. and obtained high boiling condensation products and gaseous products consisting of 15% unsaturated hydrocarbons, 30% methane, 12% carbon monoxide, 15.5% carbon dioxide, and 2% hydrogen. The presence of butadiene was not reported. By the non-catalytic pyrolysis of butanone-2, Hurd and Kocour (8) obtained small yields of ketene and methyl ketene.

Experimental

Materials

Crude *levo*-butanediol-2,3 (produced by the fermentation of wheat mashes by *Aerobacillus polymyxa*) was obtained from the Division of Applied Biology of the National Research Council, Ottawa. This material was diluted with an equal volume of water and heated on a steam-bath for several hours with 200 gm. of calcium oxide per litre of solution to remove any acetic acid

Following filtration, the greater part of the water was removed by simple distillation and the material finally fractionated under reduced pressure through a 3 ft. Stedman column. The physical constants of the purified glycol were: b.p. 177° C. (760 mm.), d_4^{20} 0.992, n_D^{20} 1.4312.

Butanone-2 (methyl ethyl ketone), obtained from the Eastman Kodak Company, was fractionated through a 3 ft. column packed with $\frac{1}{8}$ in. glass helices. The purified material had the constants: b.p. 79.6° C. (760 mm.), d_4^{20} 0.804, $n_D^{15.9}$ 1.3808.

Preparation of Catalyst

Finely powdered Morden bentonite was obtained from the Pembina Mountain Clays Ltd. of Winnipeg. It was activated according to the method of Gallay (5). The material was treated with 20% aqueous sulphuric acid of such a volume that the weight of concentrated acid was 50% of the weight of the clay being activated. This mixture was boiled gently with stirring for three hours. The slurry was diluted to three times its volume, filtered, and the clay washed thoroughly, dried at 110° C. for 12 hr., broken up, and screened to 4 to 8 mesh. After a catalysis run, the catalyst usually carried deposited carbon. This was removed and the activity restored by passing air over the catalyst at 550° to 600° C. for five to eight hours.

Apparatus and Procedure

The apparatus used is shown in Fig. 1. In operation, mercury was fed from the constant-level mercury reservoir, *A*, into a graduated chamber, *B*, containing the liquid under investigation. The rate of flow was controlled by means of a stopcock. By displacement, the liquid was forced into the preheater, *C*, consisting of a 250 cc. flask immersed in an oil-bath, which was maintained at a temperature from 30° to 50° C. above the boiling point of the liquid. The vaporized material then entered the catalyst tube, *D*, consisting of a No. 172 Pyrex resistant glass tube 100 cm. in length and 1.5 cm. inside diameter, contained in a slightly inclined electric furnace. A chromel–alumel thermocouple was placed inside the furnace and immediately outside the catalyst tube. The liquid products were condensed by means of condenser, *F*, and collected in receiver, *G*, while the gaseous products were passed through the bubbler, *I*, and into the gas-meter, *J*. A gas sample for analysis was taken, during the course of a run, into an evacuated gas sampling tube, *K*. In order to ensure equilibrium conditions, a forerun of 15 to 20 ml. was passed before collecting the liquid and gaseous products for analysis. A run usually consisted of 100 ml. of material in addition to the forerun.

Several additional experiments were carried out in the presence of water vapour. Butanediol-2,3 was diluted with the desired amount of water and passed into the preheater from one feeder. Butanone-2 and water, being only partially miscible, were fed from separate feeders. In the case of certain water-vapour runs carried out at fast feed rates, a differently designed apparatus was required to ensure complete vaporization of the material before reaching the catalyst. This apparatus consisted of a 1 in. iron pipe reactor

contained in a vertical electric furnace. The upper portion of the reactor was packed with coarse steel wool, which served as the preheater, while the lower portion was packed with the catalyst. The preheater zone was separately

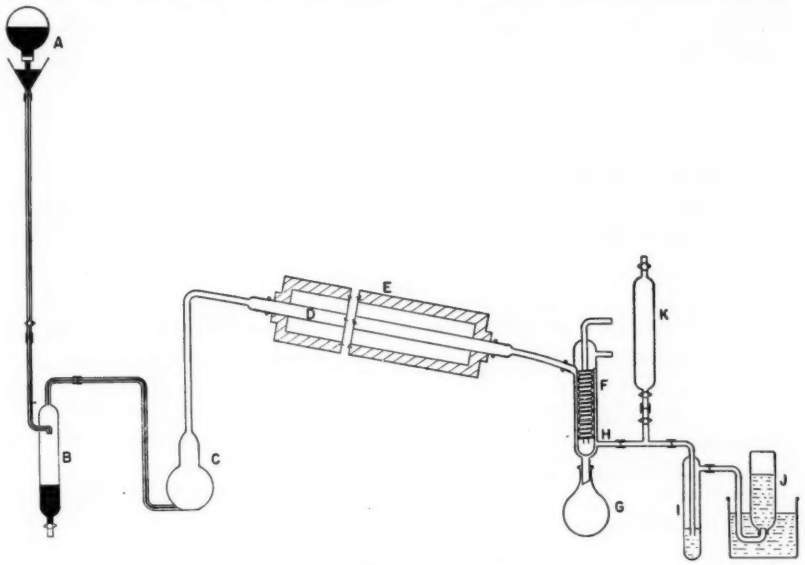


FIG. 1. *Catalysis apparatus.*

controlled and the heat input so adjusted that the vaporized material entered the catalyst zone at the desired reaction temperature. Temperatures were measured by means of two thermocouples, one placed inside the reactor, with the junction immediately above the catalyst zone, and the second placed outside the iron reactor, with the junction about two-thirds up the column of catalyst.

Analytical Methods

The liquid products consisted of two layers, an upper organic and a lower aqueous layer. The organic layer was separated, dried, and given a preliminary distillation to remove high boiling material and tarry residue. The product distilling below 150° C. was carefully fractionated through an 18 in. column packed with 3/32 in. glass helices. *p*-Cymene was used as an inert booster. Pure samples of the various components were obtained by combining similar fractions from several runs and refractionating.

The gaseous products were analysed in a modified Bone and Wheeler apparatus. The approximate composition of the gaseous olefins was determined by formation of their dibromides followed by careful fractionation under reduced pressure. *p*-Bromotoluene (b.p. 90° C. (50 mm.)) was used as the booster. The identity of the saturated hydrocarbons was determined by

passing the gaseous products, from which carbon dioxide and unsaturated hydrocarbons had been removed, through a series of cold traps. The first was maintained at −80° C. for the condensation of butane and propane, the second maintained at −110° C. for ethane, and the third trap was immersed in liquid air for the condensation of methane. Butadiene was determined volumetrically by absorption in molten maleic anhydride. The apparatus was a modification of that used by Cambron (3).

Results

Dehydration of Butanediol-2,3 in the Absence of Water Vapour

Experiments showing the effect of temperature and feed rate on the dehydration of butanediol-2,3 in the absence of water vapour are given in Table I.

TABLE I

DEHYDRATION OF BUTANEDIOL-2,3 OVER ACTIVATED MORDEN BENTONITE

Run No.	15	16	12	13	3	4	6	7	9
Temperature, ° C.	225	225	275	350	450	550	650	700	700
Feed rate, moles of glycol per hr. per 100 ml. of catalyst	0.635	1.91	1.91	1.91	0.635	0.635	0.635	0.635	1.91
Water formed, moles per mole of glycol	1.01	0.97	1.00	1.02	1.31	1.51	1.39	1.36	1.36
Vapour products, litres per mole of glycol	—	—	—	—	2.59	10.1	21.6	34.2	18.0
Main liquid products, % of theor. yield									
Isobutyraldehyde	3.0	2.6	6.5	12.1	5.7	3.6	1.4	0.5	2.3
Butanone-2	85.0	86.0	83.7	70.1	57.3	39.9	26.9	10.3	36.9
Cyclic acetal	4.7	6.2	2.8	1.7	2.2	2.2	0.8	0.5	0.7
Gas analysis, vol. %									
CO_2	—	—	—	—	17.4	7.3	1.7	1.9	2.1
CO	—	—	—	—	14.7	16.7	27.2	27.2	24.6
H_2	—	—	—	—	0.0	4.1	11.6	14.1	11.5
$CH_2 = CH_2$	—	—	—	—	2.2	3.0	5.0	8.2	6.5
Other unsaturated hydrocarbons	—	—	—	—	43.3	39.1	27.8	20.6	31.5
CH_4	—	—	—	—	3.5	10.4	12.5	17.0	14.9
C_2H_6	—	—	—	—	15.8	17.9	12.5	9.3	8.6
Butadiene in gaseous products, vol. %	—	—	—	—	5.3	4.2	4.2	4.4	7.6
Butadiene yield, % of theor.	—	—	—	—	0.8	2.0	4.1	6.7	6.1

Analysis of the organic liquid products resulted in a separation into four main fractions in addition to a tarry residue. Fraction 1, boiling at 60° to 70° C. (mainly at 64° to 66° C.) consisted of isobutyraldehyde. Fraction 2, collected at 77° to 82° C. (mainly 79° to 80° C.) was practically pure butanone-2. Fraction 3, distilling at 125° to 145° C. with a flat in the distillation curve at 137° to 138° C., consisted mainly of the cyclic acetal formed by the condensa-

tion of the glycol with isobutyraldehyde. This compound was identified by synthesis in the liquid phase and by its hydrolysis to butanediol-2,3 and isobutyraldehyde. Fraction 4, distilling at 150° to 250° C., could not be separated into any single component. It was believed to be a complex mixture of high boiling condensation products from butanone-2 similar to that found by Mitchell and Reid when this ketone is passed over silica gel (12). The physical constants obtained for the purified products were as follows:

Isobutyraldehyde	b.p. 64-65° C. (760 mm.)	d_4^{20} 0.798	n_D^{20} 1.3747
2,4-dinitrophenylhydrazone	m.p. 183-183.5° C.		
Butanone-2	b.p. 79.6° C. (760 mm.)	d_4^{20} 0.804	$n_D^{18.9}$ 1.3707
Isobutyral of butanediol-2,3	b.p. 136-139° C. (760 mm.)	d_4^{20} 0.893	n_D^{20} 1.4078

The effect of temperature upon the yields of the major liquid components is shown in Fig. 2.

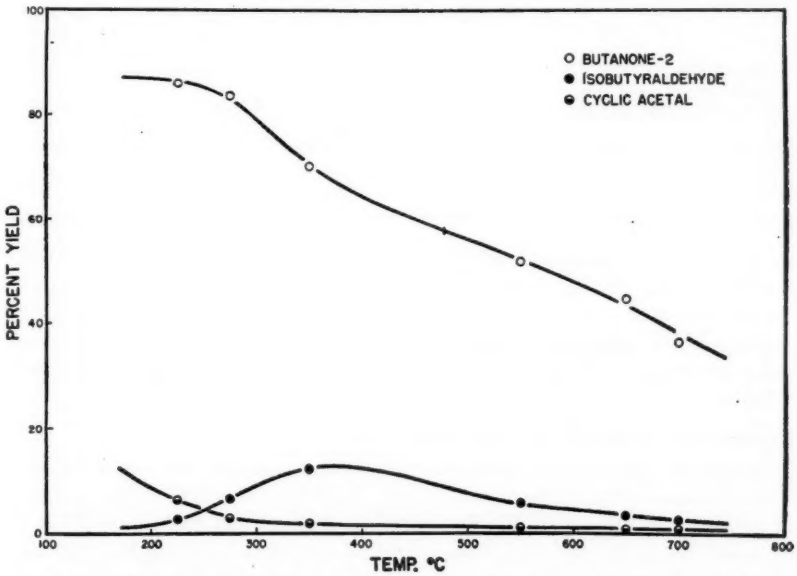


FIG. 2. *The effect of temperature on the yields of liquid products from butanediol-2,3, at a feed rate of 1.91 moles per hour per 100 ml. of catalyst.*

The identity of the olefinic hydrocarbons was determined by fractionation of the liquid dibromides formed from the gaseous products of Run 6. The approximate composition in volume per cent was found to be: ethene 12%, propene 37%, isobutene 21%, and butene-2 (*cis* and *trans*) 30%. Saturated hydrocarbons consisted of methane and ethane only, the amount of each being calculated from data obtained by combustion analysis.

Dehydration of Butanediol-2,3 in the Presence of Water Vapour as Diluent

Experiments showing the effect of temperature, feed rate, and water dilution on the dehydration of butanediol over bentonite are given in Table II.

TABLE II

DEHYDRATION OF BUTANEDIOL-2,3 OVER ACTIVATED MORDEN BENTONITE IN THE PRESENCE OF WATER VAPOUR

Run No.	14	17	75	76
Temperature, ° C.	650	700	700	700
Rate of glycol feed, moles per hr. per 100 ml. of catalyst	0.635	0.635	0.635	0.305
Rate of water feed, moles per hr. per 100 ml. of catalyst	3.18	3.18	6.36	13.71
Vapour products, litres per mole of glycol	10.1	24.5	32.7	25.5
Gas analysis, vol. %				
CO_2	2.5	1.9	5.8	3.6
CO	17.4	17.9	24.2	18.4
H_2	7.7	13.8	14.0	21.8
$CH_2 = CH_2$	5.6	6.5	3.6	3.2
Other unsaturated hydrocarbons	43.4	34.1	31.4	40.3
Saturated hydrocarbons	21.5	23.5	18.1	9.9
Butadiene in gaseous products, vol. %	13.6	7.7	11.6	20.0
Butadiene yield, % of theor.	5.8	8.4	18.8	25.3

The first two runs shown were carried out in the all-glass apparatus, while for Runs 75 and 76 the iron catalysis tube was employed. Organic liquid products were not analysed since their solubility in the large amount of water present made a separation difficult. It is seen that the presence of water greatly increased the butadiene yield, 25.3% of the theoretical being obtained in Run 76.

Decomposition of Butanone-2 Over Morden Bentonite

Experiments showing the effect of temperature, feed rate, and water dilution on the decomposition of butanone-2 are given in Table III. The vertical iron furnace was employed for the experiments involving the use of water to accommodate the faster feed rates. Liquid products were analysed in the case of the dry runs only and were found to consist mainly of water and unchanged ketone. In addition, a small quantity of isomeric pentenes and hexenes and considerable amounts of high boiling material and residue were obtained. It was found that the yield of butadiene increased with increased water dilution. A maximum yield of 44.8% butadiene based on charged ketone was obtained in Run 73, conducted at 700° C. and with a ketone–water molar ratio of 1 to 40. A lower yield in Run 74 with a ketone–water molar ratio of 1 to 60 may have been caused by fluctuations in catalyst temperature due to the fast feed rates involved. Yields based on ketone con-

TABLE III

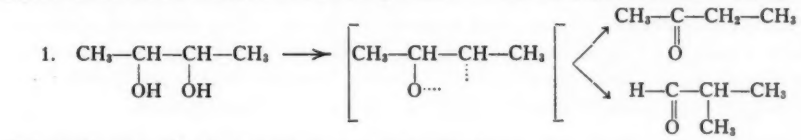
DECOMPOSITION OF BUTANONE-2 OVER ACTIVATED MORDEN BENTONITE

Run No.	21	19	22	20	23	71	72	73	74
Temperature, ° C.	450	550	550	650	650	650	700	700	700
Rate of ketone feed, moles per hr. per 100 ml. of catalyst	0.635	0.635	1.59	0.635	1.91	0.635	0.635	0.318	0.318
Rate of water feed, moles per hr. per 100 ml. of catalyst	—	—	—	—	—	3.96	8.72	12.70	19.10
Water formed, moles per mole of ketone	0.20	0.49	0.30	0.45	0.24	—	—	—	—
Vapour products, litres per mole of ketone	2.83	13.4	5.15	26.9	11.5	13.0	24.8	24.8	17.5
Gas analysis, vol. %									
CO_2	43.4	18.2	23.1	5.3	8.0	4.1	2.1	1.7	1.7
CO	12.4	15.3	17.8	15.8	17.8	16.0	18.4	13.7	16.0
H_2	0.0	4.8	1.9	13.1	5.9	5.5	9.4	12.0	16.0
$CH_2 = CH_2$	1.5	2.7	2.3	5.7	5.9	6.9	4.2	2.8	2.9
Other unsaturated hydrocarbons	25.4	28.6	27.7	21.5	21.9	39.2	43.5	52.5	42.0
Saturated hydrocarbons	15.9	30.1	26.1	37.0	35.5	25.5	19.4	15.0	19.8
Butadiene in gaseous products, vol. %	4.3	1.7	3.1	3.7	3.6	12.4	26.3	40.5	33.5
Butadiene yield, % of theor.	0.5	1.0	0.7	4.4	1.8	7.2	29.1	44.8	26.3

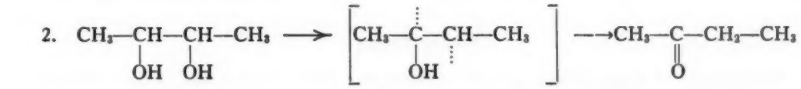
sumed were not determined owing to difficulties in separating unchanged ketone from the large volume of water present.

Discussion

Inspection of Table I shows that, at temperatures below 350° C., butanediol-2,3 is dehydrated over Morden bentonite almost entirely into the liquid products, butanone-2, isobutyraldehyde, and the isobutyral of butanediol-2,3. The carbonyl compounds result by a removal of one water molecule from the glycol followed by molecular rearrangement. This may proceed in two ways:



This is the so-called pinacolone rearrangement.



Tiffeneau has called this vinyl dehydration (16). The fact that isobutyraldehyde is also formed does not eliminate the second mechanism since both

modes of dehydration may be occurring simultaneously, the first producing ketone and aldehyde, the second forming ketone only.

Akabori (1) obtained both butanone-2 and isobutyraldehyde by distillation of the glycol with 25% sulphuric acid. He suggested that butanone-2 resulted by vinyl dehydration and isobutyraldehyde by a true pinacolone rearrangement.

The isobutyral of butanediol-2,3 is formed by the elimination of water between isobutyraldehyde and the glycol. Backer (2) obtained the cyclic acetal of butanone-2 and butanediol-2,3 by passing the glycol over alumina, while Neish, Haskell, and Macdonald (13) obtained this acetal by treating the glycol in the liquid phase with sulphuric acid.

As shown in Tables I and II, temperatures of 450° C. and higher cause a decomposition of the glycol into gaseous products. Only very small amounts of butadiene are formed when the dry glycol is passed over the catalyst, while the yield of the diolefin is increased when water vapour is used as a diluent. The question arises as to whether the high-temperature gaseous products are formed directly from the glycol or by a further dehydration of the low-temperature liquid products. From a comparison of Tables II and III, it would appear that butanone-2 is intermediate in the formation of butadiene from butanediol-2,3 since, in the presence of water vapour, the diolefin is formed in considerable quantities from both the ketone and glycol. The somewhat lower yields obtained from the glycol may be due to the formation of isobutyraldehyde and other products that are not converted into butadiene.

A two-step mechanism may be suggested for the formation of butadiene from butanone-2. The first step involves the elimination of water to form methylallene, this being followed by a rearrangement to butadiene.

$$CH_3—CO—CH_2—CH_3 \longrightarrow CH_2=C=CH—CH_3 + H_2O$$

$$CH_2=C=CH—CH_3 \longrightarrow CH_2=CH—CH=CH_2$$

Slobodin (15) passed methylallene over floridin at 330° C. and obtained 20.6% butadiene, 3.9% ethylacetylene, and 75.7% unchanged methylallene. Hurd and Meinert (9), on the other hand, have found that a non-catalytic pyrolysis of methylallene at 500° to 550° C. resulted in complete decomposition to carbon, hydrogen, ethane, and propene in 36 sec. The observed effect of water vapour in increasing the yield of butadiene from butanone-2 may be explained on the basis that catalyst contact time has been so reduced that decomposition is hindered and butadiene results by rearrangement.

As far as the authors are aware, this is the first time that the formation of butadiene by the dehydration of butanone-2 has been reported.

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BINARY AND TERNARY EUTECTICS INVOLVING AMMONIUM NITRATE[1]

By A. N. Campbell[2] and A. J. R. Campbell[3]

Abstract

With the object of preparing an 80 : 20 amatol that could be poured, instead of hand-stemmed, the eutectics of many binary and ternary systems, of which ammonium nitrate was one component, have been investigated. It was previously known that ammonium nitrate and sodium nitrate had a eutectic at 120.8° C., a temperature still too high for shell filling, but this binary mixture was frequently used as a basis for the construction of ternary mixtures. The nature of the third component was dictated by practical considerations and varied widely, e.g., alkali nitrate, nitrate of heavy metal, nitrate of organic base, and various organic substances. None of these ternary mixtures was actually used as a shell filling (with trinitrotoluene), though the ammonium nitrate–ethylenediamine dinitrate eutectic, containing 50% ethylenediamine dinitrate and melting at 100° C., comes near to satisfying all requirements; this mixture is entirely non-hygroscopic. Incidental to the work, some interesting phenomena were observed, e.g., the discovery that octadecylamine nitrate forms liquid crystals.

Introduction

The work detailed in this communication was carried out under the auspices of the Associate Committee on Explosives of the National Research Council of Canada. The essential problem was that while 40 : 60 amatols (mixtures of ammonium nitrate and trinitrotoluene) melt to a sludge at the temperature of boiling water, and thus can be readily poured, the 80 : 20 amatol, owing to its low content of trinitrotoluene, forms only a stiff mass under similar conditions, and this has to be charged into shells either by hand or mechanically; this leads to a low charge density.

Our aim was to produce, by admixture, an 80 : 20 amatol that would pour like a 40 : 60 amatol, with corresponding high density. For this purpose we used the binary eutectic ammonium nitrate–sodium nitrate as a starting point. This was shown by Early and Lowry (4) to lie at 20.5% sodium nitrate and a temperature of 120.8° C. This temperature is still too high for shell filling and therefore we studied the addition of various third substances, principally of an oxidizing character, to produce low melting ternary eutectics. For practical reasons, none of these were actually used in shell filling, but the theoretical interest attaching to these ternary eutectics remains.

Experimental

The ammonium nitrate was obtained from the Welland Chemical Works. It had a density of $d_4^{25} = 1.720$, and a moisture content of 0.304%. An analysis accompanying the consignment showed the impurities to be trifling

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in amount. The technique of the work was thermal analysis in its simplest form. The mixture, completely melted in a large test-tube, was allowed to cool freely, and temperature readings were taken every half-minute. The details of the systems investigated are given below.

Ammonium Nitrate – Trinitrotoluene

It appeared that the melting point of trinitrotoluene was unaffected by addition of ammonium nitrate, i.e., the liquids are mutually insoluble. This accounts for the formation of a sludge when an amatol is melted, the ammonium nitrate merely remaining suspended in the liquid trinitrotoluene.

Ammonium Nitrate – Alkali Nitrates

Ammonium Nitrate – Sodium Nitrate – Potassium Nitrate

This was an attempt to lower the temperature of the binary eutectic, ammonium nitrate – sodium nitrate (see Introduction) by the addition of potassium nitrate, but the effect was found to be only slight, probably owing to the formation of solid solutions, as mentioned in a previous paper (3). Thus a mixture containing 76.5% ammonium nitrate, 19.65% sodium nitrate, 3.85% potassium nitrate, froze at about 118.5° C., while one of composition 73.5% ammonium nitrate, 19.0% sodium nitrate, 7.5% potassium nitrate, froze at 117.5° C.

A third mixture of composition 72% ammonium nitrate, 20% sodium nitrate, and 8% potassium nitrate, froze at the eutectic temperature of 117.5° C. This temperature is not low enough for shell filling, but we thought this might represent a convenient method of preparing the ammonium nitrate – potassium nitrate solid solutions which are so desirable as a means of preventing the allotropic transformations of ammonium nitrate (3). We therefore investigated whether the above eutectic mixture did or did not exhibit allotropic transformation. Accordingly, a dilatometer was charged with the eutectic mixture and allowed to cool from 130° C. Allotropic transformation, no doubt III $\longrightarrow$ IV, took place at 12° C. Apparently the presence of the sodium nitrate prevents the potassium nitrate exerting its full effect. The explanation is no doubt as follows. It is known that sodium nitrate and potassium nitrate form a continuous series of solid solutions above 130° C. (1). Below this temperature the solid solution breaks up into a conglomerate but the liberated potassium nitrate will not be entirely taken up by the ammonium nitrate, since the system is now largely solid. In other words, the content of potassium nitrate in the ammonium nitrate – potassium nitrate solid solution is less than that which appears from the total potassium nitrate content, and hence the depressing effect on the transition temperature is not so great.

Ammonium Nitrate – Sodium Nitrate – Lithium Nitrate

Lithium nitrate does not form solid solutions with ammonium nitrate (2, 5), and hence this substance had a large effect in depressing the temperature of

eutectic crystallization. The lithium nitrate was dehydrated by keeping it in quiet fusion just above its melting point. The following are the results.

Composition of mixture	First appearance of crystals, ° C.	Eutectic temperature, ° C.
16% $NaNO_3$, 21% $LiNO_3$	105	83.7
18% $NaNO_3$, 20.5% $LiNO_3$	113	82.0
16.5% $NaNO_3$, 18.5% $LiNO_3$	106	83.7

The above compositions were chosen by first determining the binary eutectic lithium nitrate–sodium nitrate as lying at 47.0% sodium nitrate and 191° C., and then on a triangular diagram, joining the points corresponding to the compositions of the binary eutectics to the opposite angular points. A small triangle is thus formed whose angular points approximate to the composition of the ternary eutectic. It is apparent that none of the above mixtures is far removed from the ternary eutectic composition. The density of the (molten) mixture containing 18.5% lithium nitrate at 100° C. was found to be 1.61. Mixtures containing lithium nitrate are somewhat hygroscopic.

The Quaternary System: Ammonium Nitrate–Sodium Nitrate–Potassium Nitrate–Lithium Nitrate

A mixture of the following composition: 69.5% ammonium nitrate, 20% sodium nitrate, 8% potassium nitrate, 2.5% lithium nitrate, froze at 116° C. and solidified completely at 110.7° C.

Ammonium Nitrate–Sodium Nitrate–Nitrate of Heavy Metal

Systems Containing Zinc or Cadmium Nitrates

Zinc nitrate was found too difficult to dehydrate. The literature regarding the dehydration of cadmium nitrate tetrahydrate is confusing and inaccurate. Anhydrous cadmium nitrate is said to be stable up to its melting point, but this is not true of the hydrated salt, which undergoes hydrolysis much below that temperature (360° C.) when heated under atmospheric pressure. We eventually succeeded in dehydrating cadmium nitrate by the following procedure. The tetrahydrate was heated slowly in an oil-bath, under reduced pressure (steady pumping with a Hyvac) at 80° to 90° C. for two to four hours, for a charge of approximately 100 to 130 gm. hydrated salt. This removed about 80% of the total water of crystallization. The temperature was then raised slowly under constant suction to 140° C., over a period of about two hours; this removed a further 12% of water. Finally the mass was heated, still under suction, to 180° to 190° C. for about one hour, and this removed the last of the water. The binary system ammonium nitrate–cadmium nitrate was first investigated with the following results.

Binary System:—Ammonium nitrate – cadmium nitrate

% $Cd(NO_3)_2$	Freezing temp., ° C.	% $Cd(NO_3)_2$	Freezing temp., ° C.
10% (1)	158.5	30% (2)	119
	158.5		118
	158.0	40 (1)	97–96
10 (2)	159–155	40 (2)	96.5–94.5
20 (1)	140.4	50	98.5
20 (2)	141–137	60	111.5
30 (1)	118.5		

It was found impossible to prepare a clear solution containing 75% cadmium nitrate, as decomposition of ammonium nitrate set in at the high temperature. There are several peculiar features of this system. Although the freezing points are reasonably reproducible, no evidence of a eutectic halt was ever observed; the mixtures became solid at different temperatures, the greater the proportion of cadmium nitrate the lower the temperature. It may be that solid solutions are formed. It may also be that our preparations of dehydrated cadmium nitrate still contained moisture or basic salt, but this does not seem to be borne out by the constancy of the freezing temperatures.

A rough interpolation gives the mixture of lowest freezing point as lying at 46% cadmium nitrate and 84° C.

Ternary System:—Ammonium nitrate – cadmium nitrate – sodium nitrate

Composition		F.p., ° C.	Temp. of complete solidification, ° C.
% $NaNO_3$	% $Cd(NO_3)_2$		
12	42	117.5	113
19.7	3.85	120	117.2
19.0	7.4	119	113.5
17.1	16.65	118.5	107.0

Further work with cadmium nitrate was abandoned because it did not seem possible to produce with any mixture a sufficiently low temperature. In this regard it seems as though the addition of cadmium nitrate alone produces a lower solidification temperature than it does in the presence of sodium nitrate. There are several possible theoretical explanations but, in the absence of further experimental work, it does not seem advisable to go into this. It should be pointed out that between the freezing temperature and the indefinite 'solidifying temperature' the mixture is steadily thickening and becoming less manageable.

Systems Containing Barium Nitrate

Binary System:—Ammonium nitrate – barium nitrate

% $Ba(NO_3)_2$	F.p., ° C.
5	163
7	167
10	189–185

The binary eutectic appears to contain less than 7% $Ba(NO_3)_2$ and to freeze around 163° C., i.e., the depression in freezing point of ammonium nitrate is very slight.

Ternary System:—Ammonium nitrate – sodium nitrate – barium nitrate

Composition		F.p., ° C.	Eutectic, ° C.
% $NaNO_3$	% $Ba(NO_3)_2$		
19.5	5	160	120.3
33.0	4	160.5	120.3

Apparently the presence of barium nitrate hardly affects the eutectic temperature (120.8° C.) of the binary system: ammonium nitrate – sodium nitrate.

System Containing Lead Nitrate

Ternary System:—Lead nitrate – sodium nitrate – ammonium nitrate

Composition		F.p., ° C.	Eutectic, ° C.
% $NaNO_3$	% $Pb(NO_3)_2$		
15	27.5	129.5	111.8
29	23	168	111.5
18	14	122	111.5

Ammonium Nitrate – Sodium Nitrate – Organic Substance

Systems Containing Urea

Binary System:—Ammonium nitrate – urea

According to the literature the binary eutectic contains 48.27 mole % of ammonium nitrate and lies at 46.9° C.

Ternary System:—Ammonium nitrate – sodium nitrate – urea

% $NaNO_3$	% Urea	F.p., ° C.	Complete solidification
19.7	3.85	116	Solid at 109
15.5	24.3	89	39.5 (eutectic)
17.7	13.8	101	Solid at 82.3

Systems Containing Urea Nitrate

It was found that the mixtures containing urea were extremely hygroscopic. It was thought that urea nitrate might have a similar effect in depressing the freezing point, without the occurrence of this hygroscopicity.

Binary System:—Ammonium nitrate – urea nitrate

% Urea nitrate, 10; F.p., ° C., 148.

Ternary System:—Ammonium nitrate – sodium nitrate – urea nitrate

Composition		F.p., ° C.
% $NaNO_3$	% Urea nitrate	
19.7	3.85	119
19.7	3.85	118
19.0	7.4*	117
17.65	13.75*	114
16.5	19.3*	109.5
15.5	24.2*	108.5
18.7	7.45*	117.2
17.7	13.8*	114
16.6	19.4*	109.5
15.5	24.3*	108.5

* *All these mixtures effervesce in the liquid state.*

System Containing α-Nitronaphthalene

A molten mixture containing 10% α-nitronaphthalene (90% ammonium nitrate) deposited crystals at 168.5° C. and was sensibly solid at 168.0° C., i.e., molten ammonium nitrate and molten α-nitronaphthalene are mutually insoluble.

Experiments on Hygroscopicity

So many of these mixtures appeared to be highly hygroscopic that a systematic investigation of the matter was undertaken. About 25 gm. of each substance or mixture was exposed to the atmosphere in an open dish and weighed from day to day. The results are contained in the following table. (The weather during the experiments was decidedly humid.)

Substance or mixture	Duration of experiment, days	% H_2O absorbed
1. *NH_4NO_3 alone (dried over H_2SO_4 for a year)	28	0.0
2. $NaNO_3$ alone	28	0.0
3. Urea alone	28	0.0
4. $Cd(NO_3)_2$ anhyd. alone	25	12.55
5. NH_4NO_3–$NaNO_3$ (binary eutectic)	22	9.8
6. NH_4NO_3–$Cd(NO_3)_2$ (binary eutectic)	19	37.7
7. NH_4NO_3–$NaNO_3$ Urea (ternary eutectic)	28	20.9
8. NH_4NO_3–$NaNO_3$–$Pb(NO_3)_2$ (ternary eutectic)	26	16.2
9. NH_4NO_3–$NaNO_3$–$LiNO_3$ (ternary eutectic)	25	29.2
10. NH_4NO_3–$NaNO_3$–KNO_3 (ternary eutectic)	21	11.05
11. NH_4NO_3–$NaNO_3$–$Cd(NO_3)_2$ (ternary eutectic)	19	41.7

* *This result is surprising in view of the notorious hygroscopicity of ammonium nitrate. During the first part of the month, when the weather was very humid, the ammonium nitrate did increase in weight by 0.085%, but it lost this as the atmosphere dried up again. We are inclined to doubt whether absolutely pure ammonium nitrate is hygroscopic. It has always been thought to be so, but only since the advent of the Haber and similar processes has really pure ammonium nitrate been obtainable commercially.*

It is noteworthy that while the components themselves are not appreciably hygroscopic (with the exception of cadmium nitrate), the binary eutectics are very hygroscopic, and the ternary eutectics still more so. This is connected with the lowering of the melting point.

Systems Containing Phenol

Phenol is known to form a compound with urea having the composition 2 moles phenol: 1 mole urea, and this compound, having an incongruent melting point, is completely dissociated in the liquid state. It was thought that the dissociated urea might, in the liquid state, exert its powerful depressing effect on the melting point of ammonium nitrate, while, after solidification, the phenol might unite with the urea to form a non-hygroscopic mixture. The first question to investigate was whether this compound of 2 phenol: 1 urea was itself hygroscopic. So far from being hygroscopic, the compound, on prolonged exposure to the atmosphere, lost weight slowly but steadily, probably owing to volatilization of the phenol.

Binary System:—Ammonium nitrate – 2 phenol: 1 urea

% Phenol–urea	F.p., ° C.	Eutectic
10	159.5	All solid at 145.0° C. except for layer of liquid phenol.
55	115	Lower layer solid at 105° C.

This device failed because the gravitational separation of the dissociated phenol gave rise to two liquid layers.

Ammonium Nitrate – Ethylenediamine Dinitrate

The purity of the ethylenediamine dinitrate was estimated by distillation with sodium hydroxide and collection of the distillate in standard acid, followed by back titration with standard alkali. Purity = 99.5%.

Equilibrium Diagram. System:—Ammonium nitrate – ethylenediamine dinitrate

Wt. % E.D.D.	Freezing point, °C.	Eutectic temperature, ° C.
10	151	Solid at 132
20	137	Allotropic transformation at 124; stirring ceased at 105
30	122	97.3
40	110	97.3
50	98.2	96.8
50 (repeat)	100.2	103
	101.7	104
60	112.5	102.1
80	135.5	Solid at 124.5
90	148	Obvious decomposition of ethylenediamine dinitrate.

The thermal determination of the eutectic temperature is seen to be rather hazy. When the freezing point curves are plotted, the diagram is seen to be of simple eutectic form (absence of compound formation), the eutectic lying at 50% ethylenediamine dinitrate (by weight) and about 100° C. A direct investigation of a freshly prepared 50% ethylenediamine dinitrate gave crystals at 99.0° C., followed by a rise to constant temperature at 102.5° C. This therefore may be taken as the true eutectic temperature.

Hygroscopicity of Ammonium Nitrate – Ethylenediamine Dinitrate Eutectic

After 18 days' exposure to the atmosphere, the increase in weight amounted to 0.0089%.

The System:—Ethylenediamine Dinitrate – Trinitrotoluene

The melting point of trinitrotoluene is unaffected by the presence of ethylenediamine dinitrate; in other words, the liquids are mutually insoluble.

Effect of Ethylenediamine Dinitrate on Allotropic Transformations of Ammonium Nitrate

It was possible, despite the eutectic form of the equilibrium diagram, that ethylenediamine dinitrate might form solid solution with ammonium nitrate and thus affect its transition points. Accordingly, this point was investigated dilatometrically.

A mixture of 10% ethylenediamine dinitrate and 90% ammonium nitrate was melted at 180° C., poured into a mortar, cooled, and ground. Thirty-five grams of this mixture was placed in a dilatometer having a wide capillary so that the whole range of transitions could be investigated without refilling. The indicator fluid was xylene. The completely filled dilatometer was heated at 120° C. for half an hour to ensure equilibrium. The temperature was then allowed to fall, the position of the meniscus being followed, to room temperature. The measurements were then repeated with rising temperature.

1. *Falling temperature* — *Transitions at:—*
   66.5° C. II $\longrightarrow$ III
   27.5° C. } III $\rightleftharpoons$ IV
2. *Rising temperature* — 37.0° C. } Mean = 32.25° C.

From this it is concluded that ethylenediamine dinitrate has no effect on the transitions:—IV $\rightleftharpoons$ III and III $\rightleftharpoons$ II. The thermal analysis showed that the transformation II $\rightleftharpoons$ I, at 124° C., is not affected. Hence ethylenediamine dinitrate does not form solid solutions with ammonium nitrate.

Density of solid ethylenediamine dinitrate at 25° C.

$$d_4^{25} = 1.598, 1.593: \text{mean} = 1.595$$

Density of solid eutectic (50% ethylenediamine dinitrate)

$$d_4^{25} = 1.631, 1.635: \text{mean} = 1.633$$

*Cyclohexylamine Nitrate**

Equilibrium Diagram:—Ammonium nitrate – cyclohexylamine nitrate

10% Cyclohexylamine Nitrate. This mixture decomposed with charring below its melting point.

20% Cyclohexylamine Nitrate. Mixture decomposed below its melting point. When it was heated all night at 110° C. there was no sign of melting. The pure cyclohexylamine nitrate also decomposed at its melting point. The density was determined as $d_4^{25} = 1.204$.

Octadecylamine Nitrate

The behaviour of this substance on melting is very peculiar. Heated at the temperature of boiling water, it shrank to a paste. When the temperature was then raised to 150° C., the paste expanded to form a rubber-like mass, which became slightly yellow on cooling. We suspected the formation of liquid crystals, and accordingly an examination was made with a polarizing microscope on a heated substage. Under polarized light, there appeared to be slight movement of the crystals at 71° C.; at 76.4° C., portions of black field then appeared, interspersed with crystalline fragments. The interference persisted at 88° C. Under ordinary light at this temperature, the molten masses still showed an irregular outline, reminding one of crystals. The substance decomposed at 160° C. On cooling the isotropic liquid, interference reappeared between 70° to 90° C., in different experiments. I suggested to Dr. Downing that further investigation along these lines should be undertaken by the Crystallographic Department at Cornell. This was done and Dr. Blomquist reported as follows, in qualitative agreement with our findings.

"The compound *n*-octadecylamine nitrate which you so kindly sent us has proved to be extremely interesting. We have made some studies of the material on the hot stage microscope and by fusion analysis and have observed some interesting crystal properties. The substance exists in two solid polymorphic forms and in a liquid crystal form. All of the polymorphic transformations are reversible by temperature variation.

"On melting at 80° C. liquid crystals are formed. These appear between crossed Nicols as feathery crystals but are invisible in the liquid in the ordinary light. On making the liquid flow by moving the cover glass, the liquid crystals change shape and appearance. By pushing down on the cover glass the liquid crystals line up so that one looks down BX_a, thus the interference figure has been obtained and shows the optic axial angle, 2V, to be about 15° with slight dispersion.

"On cooling, the liquid crystals transform to a true solid modification at about 80°. The transformation is reversible. This solid modification shows very low birefringence. Mottled areas of random crystal orientation show first order grey and white. A few areas show an imperfect optic axis interference figure. 2V is in the range 70° – 90° C.

* *We are indebted to Mr. E. Pritchard for the experimental work on cyclohexylamine nitrate.*

"Further cooling to 70° brings about another reversible transformation to the low temperature stable modification. This is a solid-solid transformation. The low temperature form appears as feather-like interlaced crystals having higher birefringence than the first solid. The small crystal size and the interlacing of other crystals make it possible to obtain good interference figures."*

Equilibrium Diagram:—Ammonium nitrate–octadecylamine nitrate

Pure octadecylamine nitrate: Freezing point = 76.1° C. (very indeterminate because of the rubber-like nature of the mass).

10% Ammonium nitrate F.p = 74.0° C. (very approximate)

20% Ammonium nitrate F.p. = 73.0° C. (uncertain)
72.3° C.

40% Ammonium nitrate F.p. = 72.3° C.

There was no indication of eutectic freezing in any of the above melts. We deduce that the solubility of ammonium nitrate in molten octadecylamine nitrate is slight.

A mixture of trinitrotoluene and octadecylamine nitrate containing 10% octadecylamine nitrate froze at the freezing temperature of pure trinitrotoluene, viz. 79.3° C. Hence, octadecylamine nitrate is insoluble in molten trinitrotoluene.

Density: $d_4^{25} = 1.044$.

Further work was abandoned because (i) octadecylamine nitrate and ammonium nitrate are mutually insoluble, or almost so; (ii) the rubber-like nature of the molten mass renders it difficult to handle; (iii) the density is extremely low.

Summary

The composition and temperature of the different eutectics are given in the following table.

System	Eutectic temp., ° C.	Eutectic composition
NH_4NO_3–T.N.T.	Same as m.p. of T.N.T.	Mutually insoluble
NH_4NO_3–$NaNO_3$	120.8	20.5% $NaNO_3$ (4)
NH_4NO_3–$NaNO_3$–KNO_3	117.5	Unknown because of formation of solid solution.
$NaNO_3$–$LiNO_3$	191	47.0% $NaNO_3$
NH_4NO_3–$NaNO_3$–$LiNO_3$	83.7	16.5% $NaNO_3$, 18.5% $LiNO_3$
NH_4NO_3–$Cd(NO_3)_2$	84†	46% $Cd(NO_3)_2$†
NH_4NO_3–$Ba(NO_3)_2$	160	Less than 7% $Ba(NO_3)_2$
NH_4NO_3–$NaNO_3$–$Ba(NO_3)_2$	160	Less than 5% $Ba(NO_3)_2$
NH_4NO_3–$Pb(NO_3)_2$	111.5	Not known
NH_4NO_3–Urea	46.9	48.27 mole % NH_4NO_3
NH_4NO_3–$NaNO_3$–urea	39.5	Not known
NH_4NO_3–$NaNO_3$–urea nitrate	108.5†	15.5% $NaNO_3$, 19.3% urea nitrate†
NH_4NO_3–ethylenediamine dinitrate	100	50% ethylenediamine dinitrate

† *Very approximate.*

* *Dr. Blomquist asks us to state that "the crystallographic work was actually carried out by Dr. W. C. McCrone, who was working under my direction."*

The above table does not contain all the systems investigated, because, for experimental reasons such as decomposition, formation of solid solutions, etc., it was not always possible to evaluate the eutectic conditions.

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MEASUREMENTS OF CURE OF SOME THERMOSETTING RESINS[1]

By Manuel N. Fineman[2] and Ira E. Puddington[3]

Abstract

The rate and degree of cure of resorcinol–formaldehyde resins and unsaturated polyesters are determined by measuring changes in their electrical resistance and density during the process of cure. Data thus obtained are interpreted in terms of the rate and extent of cross-bonding in these thermosetting resins.

Introduction

In a previous publication (1), a method for determining the rate and extent of cure for thermosetting resins of the cast phenolic type was described. This method consisted eassentially in measuring changes in the electrical resistance of the resin during the curing cycle. Since, on curing, thermosetting resins change from liquids of low viscosity to gels and finally become three-dimensional in structure, their electrical resistance increases over a very wide range until, as solids, the resins become good dielectric materials. Data thus obtained were correlated with measurements of changes of density with time of cure.

The electrical measurements were shown to be sensitive to changes in the internal molecular arrangement of these systems, such as rate and extent of cross-linkage formation, while the density determinations were mainly charateristic of macro properties. The increased rate of cure as a result of rising temperature or added accelerator and retarding effect of plasticizers were indicated by the results obtained.

In the present paper an attempt has been made to apply this procedure to other thermosetting resins such as resorcinol–formaldehyde and unsaturated polyesters. The results obtained with these materials are reported below and confirm the usefulness of this procedure in indicating experimentally the rate and extent of cross-bonding in thermosetting resins of this type.

Experimental

Preparation of Samples

Resorcinol–formaldehyde resin was prepared by mixing 125 gm. of formaldehyde (37%, U.S.P.) with 110 gm. of resorcinol (U.S.P.) kindly supplied to us by the Pennsylvania Coal Products Co. of Petrolia, Pa. These quantities represent a molar ratio of approximately 1 : 1.5 of resorcinol to formaldehyde, and the solution had a pH of about 3.0. By careful addition of small

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quantities of a sodium carbonate solution to this mixture, two other resin solutions were prepared with a pH of 5.5 and 7.0, respectively.

The unsaturated polyester used in these experiments was a commercial product of the American Cyanamid Co. known as Laminac 4122. It is an almost water-white liquid thermosetting resin, which is 100% reactive. As it is chemically unsaturated, a small quantity of peroxide catalyst was used to activate its rapid conversion to a solid, which proceeds without the formation of by-products. The catalyst used was technical lauroyl peroxide distributed by the Lucidol Corp. under the trade name of Alperox C.

Apparatus

The cells in which the resistance measurements were made were constructed of concentric copper tubing, the resin being poured into the annular space between these tubes. A General Radio Capacitance Bridge, Type 716-B, was used for the electrical measurements. For the density determinations, glass dilatometers were constructed using mercury as the displacement liquid and a horizontal capillary for measuring the changes in volume as the resin cured. This apparatus has already been described in detail (1).

Results

Fig. 1 shows the results obtained on curing resorcinol–formaldehyde resin at room temperature (i.e., 25° ± 2° C.). Plotting electrical resistance logarithmically against time of curing, Curve *A* represents the behaviour of a resin of pH 3.0; Curve *B*, of pH 5.5; and Curve *C*, of pH 7.0. The points that deviate from the curves are due to fluctuations in the room temperature that were later eliminated in the density experiments by the use of a thermostatically controlled water-bath. The resin of pH 7.0 cured very rapidly immediately after formation, with the evolution of a large quantity of heat which further speeded the reaction, causing boiling inside the resin and the formation of a very porous solid. In order to prevent too rapid cure, the resin represented by Curve *C* was cooled by running water for the first hour after preparation and then poured into the resistance cell. Thereupon cure took place at a more moderate rate, continuing for several days. Fig. 1 shows that the slope of the curve and extent of change of the electrical resistance increase very rapidly as the pH of the resin is increased.

Fig. 2 shows the behaviour observed for the polyester casting resin, Laminac 4122, when cured in a constant temperature oil bath at 80° ± 0.1° C. Curve *A* represents the behaviour of the polyester with 0.5% of added catalyst; Curve *B*, with 0.3%; and Curve *C*, with 0.1%. The initial rapid drop common to the three curves represents the drop in resistance as the samples warmed up from room temperature to the temperature of the oil-bath. Then, for the first few hours of cure, the electrical resistance increased very rapidly as the samples quickly changed from a liquid of about 200 centipoises viscosity to a hard solid. Subsequently the graph shows that the electrical resistance

of the resin with 0.1% of added catalyst increased much less with time than that of either of the two other samples whose behaviour was almost identical.

The resistance data reported in Figs. 1 and 2 are for samples of 57.0 gm. weight, measured in cells whose dimensions were identical, as previously described (1). Thus the values obtained may be assumed to be directly comparable even though they are not reduced to read in terms of specific resistance.

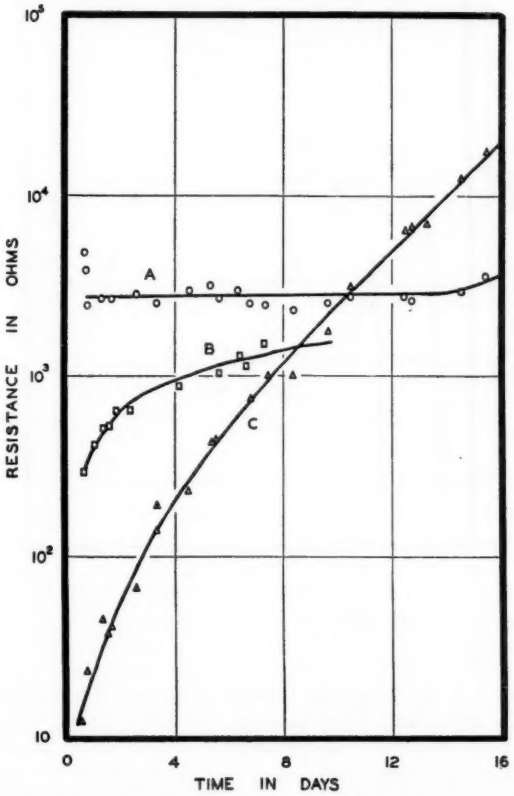

FIG. 1. *Change of electrical resistance with time of curing. Curve A, resorcinol–formaldehyde resin of pH 3.0; Curve B, of pH 5.5; and Curve C, of pH 7.0.*

In Fig. 3 the density data are reported for both the resorcinol–formaldehyde and the polyester resins. For these experiments the dilatometers were immersed in constant temperature baths that did not vary more than ± 0.1° C. The temperature of cure was 25.0° C. for the resorcinols and 80.0° C. for the polyester resin.

Curves *A* and *B* represent the changes in density with time of cure for the resorcinol resins of pH 3.0 and 5.5, respectively. Despite the marked

difference in their electrical behaviour as shown by Curves *A* and *B* of Fig. 1, the density data show that the resin of higher alkalinity displays only a slightly greater degree of shrinkage than does that of lower alkalinity. After about five to eight days of curing, these resins started to exude from 5 to 10% of water, thus making subsequent density calculations inaccurate. The resorcinol sample of pH 7.0 cured too rapidly to give accurate results in the dilatometer.

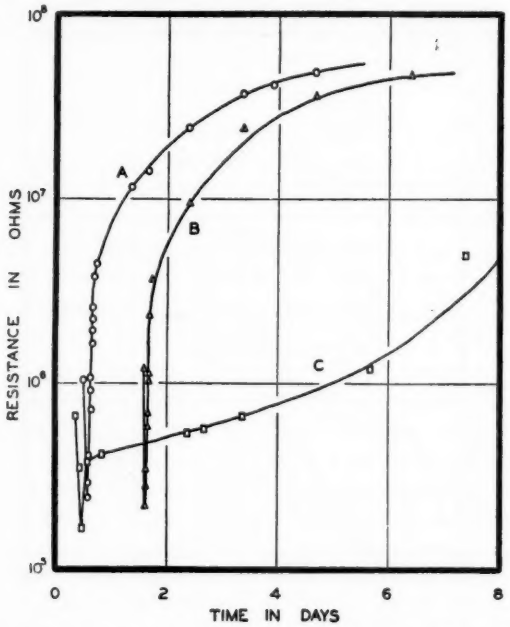

FIG. 2. *Change of electrical resistance with time of curing. Curve A, polyester with 0.5% of added catalyst; Curve B, with 0.3%; and Curve C, with 0.1%.*

Curve *C* of Fig. 3 describes the density change observed for a polyester sample at 80° C. to which 0.1% of catalyst had been added. It will be noted from the scale of the abscissa that the density change takes place very rapidly after an initial time lag—much more rapidly than in the case of the resorcinols. This time lag was partly the time required to bring the sample up to the temperature of the bath.

Where this curve shows its greatest slope, two regions are indicated over which low density values were recorded. This was probably due to the formation, at the mercury surface, of a resin-to-glass seal that did not rupture until considerable shrinkage had taken place in the body of the resin, thus preventing the accurate recording of the density change. Polyesters with a catalyst concentration of 0.3% or higher shrank even more rapidly than the sample whose density behaviour is here described. Hence no reliable data for these were obtained.

Discussion

Assuming that the passage of current through these samples depends upon the free mobility of any ions that may be present in the system, steric factors, such as the formation of cross-bonds, might be expected to restrict this property (2, p. 168). Therefore, the electrical resistance of these samples will increase continuously as the number of cross-linkages in the system increase.

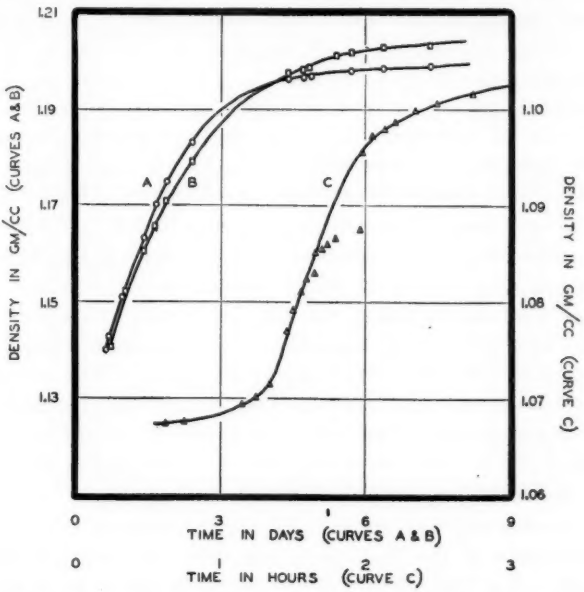

FIG. 3. *Change of density with time of curing. Curves A and B, resorcinol–formaldehyde resin of pH 3.0 and 5.5, respectively; Curve C, polyester with 0.1% of added catalyst.*

Hence the rate and extent of cross-bonding, which represents the rate and degree of cure of these materials, can be compared by measuring the changes in the electrical resistance of the samples as condensation or polymerization proceeds.

Bearing this mechanism in mind, Curve *A* of Fig. 1 would seem to indicate that very few, if any, cross-bonds are formed in a resorcinol resin of low pH. However, as the alkalinity of the resorcinol resin increases, so too does the rate of cure and the number of cross-linkages formed.

The curves of Fig. 2 can also be interpreted on this basis on the assumption that 0.1% of peroxide catalyst is not sufficient to give 100% reaction during the time of observation. Hence Curve *C* shows a much lower electrical resistance than that attained by the two other samples. In each of the latter, cross-linkage formation must have been virtually complete and hence the two curves are almost identical in shape. It may be noted that 0.3% of

catalyst is the amount recommended by the manufacturer for use under the conditions of this experiment.

With the setting up of cross-bonds, the molecules of the resin become oriented more closely together in space and occupy a smaller volume. This behaviour is demonstrated by Curves *A* and *B* of Fig. 3, which describe the curing of the resorcinol resins. As previously shown by the electrical measurements, it will be noted that here, too, the density change is slightly greater for the resin of higher alkalinity. According to the more recent theories governing the mechanism of condensation of phenols and resorcinols with formaldehyde, it has been generally accepted that, for a given resin in which normally very few cross-linkages are set up, this number can be increased by increasing the concentration of hydroxyl. In alkali, methylol groups are formed that assist in the formation of cross-linkages (3, 4). A similar case involving phenol–formaldehyde resin of molar ratio 1 : 0.8 has already been discussed (1).

Curve *C* of Fig. 3 shows that the greatest change in density for the polyester sample takes place during the first few hours of cure; this corroborates the electrical data (Curve *C*, Fig. 2), which indicate that the largest change in electrical resistance occurs during the first few hours. Moreover, although these samples may reach what appears to be a maximum density after the first few hours of cure, the electrical curves indicate that polymerization continues for several days until the maximum number of cross-bonds possible for the system are set up. This is further suggested by the fact that both Curves *A* and *B* of Fig. 2 become asymptotic to the abscissa at about the same value of the resistance, i.e., about 5×10^7 ohms.

It might be supposed that the shrinkage of the resin on curing may have caused deterioration of the contacts at the resin–electrode interface in the cylindrical cell. Any such deterioration could give rise to an increase in the measured electrical resistance. To investigate this further, a sample of the polyester was cured at 80° C. with added catalyst, using two probe electrodes immersed in the resin, instead of the cylindical cell. In this case, shrinkage of the resin could only improve the contacts. The results observed were analogous to those obtained when the cylindical type of cell previously described was employed. This has also been confirmed in the case of resol resins (1). Furthermore, it should be pointed out that the resorcinol resin of pH 3.0, which showed a marked shrinkage during the first five days of cure (Curve *A*, Fig. 3), did not show a rise in electrical resistance until after 15 days (Curve *A*, Fig. 1). These observations seem to confirm the view that the electrical data observed were not a result of deterioration at the resin–electrode interface.

It might also be suggested that loss of water by the resin during the curing process gave rise to the changes in electrical resistance that were observed. However, in the case of the polyester resin, 100% reaction is said to be obtained without the formation of by-products. That is, the curing process was one of polymerization, not condensation, and hence no water was present.

Thus it may be concluded that the changes in electrical resistance and density shown by thermosetting resins during cure are real functions of the degree of advancement of these resins and may be interpreted in terms of the mechanism of cross-linkage formation.

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CATALYTIC AROMATIZATION OF TURNER VALLEY CRUDES[1]

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Abstract

The maximum yield of toluene resulting from catalytic aromatization of a selected cut from Turner Valley crude oil has been determined. Using a catalyst containing 14% chromium trioxide on aluminium trioxide and a temperature of 515° C., a total yield of toluene equal to 3.15% by weight of the original crude may be obtained. This is 137% greater than the quantity of toluene originally present in the crude (1.33%). A possible recovery of approximately 250,000 lb. of toluene per day is indicated for the whole Turner Valley field (1942).

Introduction

It is a well known fact that North American crude oils contain a relatively small percentage of aromatic hydrocarbons (18, 27). Fortunately however, these same crude oils contain appreciable amounts of aliphatic hydrocarbons which may be catalytically converted to aromatic hydrocarbons.

A rough calculation on one of the Turner Valley crudes (Foundation well No. 1; see (27)) indicates, for example, that heptane and 2- and 3-methylhexane are present in sufficient quantity to yield 2.9% toluene (based on the original crude) by catalytic aromatization. The original toluene content of this particular crude was 1.07%, and thus an increase in the toluene yield of about 160% might be expected to result from an efficient catalytic operation.

In recent years a number of studies have been made on the catalytic conversion of aliphatics to aromatics (see, for example, 3, 6, 9, 13, 15–17, 19–21, 23–25). From these studies it appears that:

(1) A number of oxides act as efficient aromatization catalysts, chromium trioxide and vanadium pentoxide being the most active;

(2) The oxides are usually supported on alumina;

(3) Mixed oxides of chromium, molybdenum, and vanadium, on alumina, give a catalyst superior to chromium trioxide on alumina;

(4) A high conversion of aliphatics to aromatics is favoured by a temperature of about 500° C. and a low feed rate;

(5) Hydrocarbons whose structure permits direct formation of a six carbon ring are aromatized to a marked extent, whereas hydrocarbons whose structure does not permit direct formation of a six carbon ring are not appreciably aromatized.

Considerable increases in the yields of aromatics from petroleum cuts as a result of catalytic aromatization have already been reported (14, 16, 28) and

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it was thought worth while to do similar work on Turner Valley crudes, particularly as information concerning the aromatic content of some Turner Valley crudes was already available in this laboratory (11).

Experimental

Apparatus

The apparatus is illustrated in Fig. 1 and is similar to that described by Hoog, Verheus, and Zieuderweg (13). The liquid feed was vaporized at 200° C. in a preheater and the vapour passed over the catalyst. The catalyst

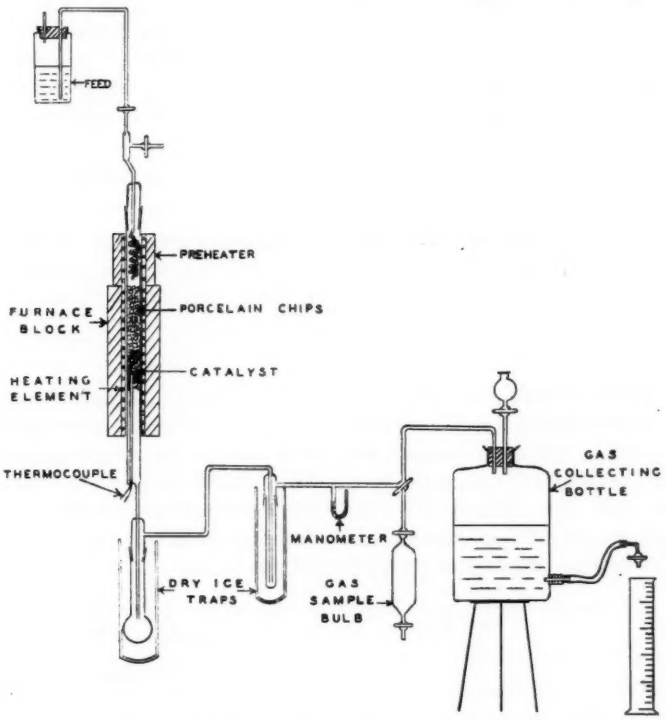

FIG. 1. *Apparatus for cyclization.*

was contained in a Pyrex tube heated in a vertical block furnace to 500° C. The product was condensed in a trap immersed in a dry-ice–acetone mixture. Any uncondensed gas was collected in a gas holder. A sample of the gas was usually analysed.

Catalyst

Catalysts 1 and 2. The catalyst was prepared as outlined by Grosse, Morrell, and Mattox (9). Eight-mesh granules of activated alumina (Alum-

inum Co. of America) were impregnated with chromium nitrate solution, dried for several hours at 100° C., and finally heated to 400° C. for four hours.

Catalyst No. 1. 4.5% Chromium trioxide on activated alumina.

Catalyst No. 2. 7.0% Chromium trioxide on activated alumina.

Catalyst No. 3. Aluminium trioxide was prepared according to Adkins, (1, 2).

Aluminium nitrate nonahydrate (300 gm.) was dissolved in 2 litres of water. The solution was heated to boiling and concentrated ammonium hydroxide solution added until precipitation was complete. The solution was diluted to 4 litres, heated to boiling for 15 min., and allowed to cool. The precipitate was washed with distilled water and centrifuged. The precipitate of aluminium hydroxide was dried, first at 100° C. and then at 500° C. to give alumina. The dried particles were about 4 to 8 mesh in size. These particles were impregnated with chromium nitrate solution to give a catalyst containing 8.3% chromium trioxide.

Catalyst No. 4. 9.7% Chromium trioxide on activated alumina.

Catalyst No. 5. 14.2% Chromium trioxide on activated alumina.

The catalyst was usually revivified after each experiment. The procedure was to flush with nitrogen, then with air at 500° C. for three hours, then with nitrogen, then with hydrogen for three hours, and finally with nitrogen (8, 22, 25).

Analysis

(a) Liquid Produc

The liquid product was analysed for aromatics by the dispersion method (10, 11, 26). The base values were checked from time to time and the dispersion method itself was rechecked with the nitration method (4) on several toluene cuts (b.p. 88° to 119° C.) from Turner Valley crudes. The base value of the aromatized material was determined in the usual way and was found to be the same as for the original feed.

Sample	Toluene in toluene cut, %	
	By nitration	By dispersion
Home Millarville 2	12.9	13.8
Royalite 31	18.8	18.8
Royalite 43	20.3	20.5

The liquid products were also analysed for olefins by the Francis method (7). The method was tested on pure cyclohexene, prepared by dehydration of cyclohexanol on activated alumina (12, p. 27). Bromine numbers of 194.9 and 193.6 were obtained; theoretical, 194.

Conjugated diolefins were tested for by the Diels–Alder reaction following the procedure of Grosse and Wackher (10). No significant amounts of con-

jugated diolefins were found, in agreement with results in similar cyclization experiments performed elsewhere.

(b) Gaseous Products

The gaseous products were analysed for carbon dioxide, oxygen, unsaturates, hydrogen, and carbon monoxide, on a modified Orsat apparatus (5). A typical gas analysis is recorded below, together with one by Taylor and Turkevich (24). Both runs were made with heptane at 515° C. using a chromium trioxide catalyst:

Constituent	Gas analysis, volume, %	
	Run No. 31	T. and T.
CO_2	0.0	0.12
Unsaturates	0.9	0.84
O_2	0.3	0.23
H_2	86.5	87.6
CO	0.6	0.73

Materials

The following materials were used in catalytic dehydrogenation experiments:

I. Hexane (Eastman), Pract.; b.p. 62° to 68° C. $d_4^{20} = 0.6621$; $n_d^{20} = 1.37567$.

II. Heptane (Eastman), Pract.; b.p. 92° to 96° C. $d_4^{20} = 0.7158$; $n_d^{20} = 1.40232$.

The heptane contained a small percentage of aromatics, which was removed by nitration. After nitration n_d^{20} was 1.39678. A true boiling curve of the heptane indicated that this material actually contained less than 40% heptane. However, it was thought that this material would show a behaviour somewhat similar to that for the toluene cut (90° to 120° C.) from Turner Valley crude, and consequently it was used in preliminary experiments to determine the effect of feed rate, temperature, and catalyst composition on the yield of aromatics.

III. Heptane (Eastman), pure; $n_d^{20} = 1.38776$.

In order to determine possible yields of toluene from Turner Valley crudes, toluene cuts were used which had been freed from toluene by nitration.

IV. Nitrated Toluene cut *(a)* from Turner Valley crudes.

One-litre portions of crude from three Turner Valley wells were fractionated in a 3 ft. Stedman column. The toluene cuts (b.p. 90° to 120° C.) were nitrated to remove any aromatics. The nitrated portions were washed and dried and used as feed in Runs 9, 12, and 13 (see Table IV).

V. Nitrated toluene cut *(b)* from Turner Valley crudes.

Thirteen litres of Turner Valley crude oil (equal volumes of Foothills 6, Home Millarville 2, and Royalite 31) were distilled in a 2-in. steel column. The cut from 70 to 130° C. was retained (1650 cc.). This cut was distilled

in a 5 ft. Stedman column (1-in. diameter), product being removed at 20 cc. per hour. The cut from 90° to 120° C., hereinafter called the toluene cut, was collected; it measured 1066 cc. From previous experience, we can say that this cut contains all the toluene, together with the hydrocarbons which will cyclize to toluene (without cracking). Four hundred cubic centimetres of this cut was nitrated to remove aromatics (called nitrated toluene cut) and was used in aromatization runs Nos. 45 to 56 (see Table VI).

The following data, obtained on the toluene cut, indicate that the original crude contained 1.33% toluene by weight.

	Density	$\delta^{20}_{g\text{-}e}$*	Aromatics (dispersion), %
Before nitration	0.7448	118.9	17.9
After nitration	0.7211	100.4	0

Aromatics by nitration, % = 17.6 Density or original crude = 0.823

$$\text{Toluene in original crude} = \frac{1066 \times 0.7448 \times 0.179 \times 100}{13000 \times 0.823} = 1.33\% \text{ (by weight)}$$

* $\delta^{20}_{g\text{-}e}$ *is the dispersion (11).*

Results

Preliminary experiments were performed in which feed rate, temperature, and catalyst composition were varied.

Variation of Feed Rate

Increasing the feed rate decreases the conversion very markedly (see Tables I and II). In the following tables, feed rates are given in cubic centimetres of liquid per hour per gram catalyst.

TABLE I

VARIATION OF FEED RATE. HEPTANE, PRACT. CATALYST NO. 2

Expt. No.	Temp., °C.	Feed rate, cc./gm. catalyst/hr.	Vol. gas, cc./gm. feed	Gas analysis, volume %					Aromatics, based on original feed, %
				CO_2	O_2	Unsaturates	H_2	CO	
19	475	0.72	180	0.6	0.8	0.3	88.5	0.8	5.2
20	475	0.27	356	0.2	1.5	0.2	83.5	0.1	9.5

Variation of Temperature

Increasing the temperature from 470° to 515° C. resulted in a considerable increase in conversion to aromatics (see Table III). A similar temperature effect may be noted in later tables (at higher temperatures the formation of coloured and gum producing products becomes pronounced).

TABLE II

VARIATION OF FEED RATE. HEPTANE, PURE. CATALYST NO. 4

Expt. No.	Temp., °C.	Feed rate, cc./gm. catalyst/hr.	Vol. gas, cc./gm. feed	Recovery, %	Aromatics based on original feed, %	Br No.
43	510	0.14	183	89.5	23.0	25.2
44	516	0.06	594	76.5	47.6	23.4

TABLE III

VARIATION OF TEMPERATURE AND CATALYST. FEED: HEPTANE, PRACT.

Expt. No.	Temp., °C.	Feed rate, cc./gm. catalyst/hr.	Vol. gas, cc./gm. feed	Gas analysis, volume %					Aromatics, based on original feed, %
				CO_2	O_2	Unsaturates	H_2	CO	
Catalyst No. 2									
29	470	0.27	133	0.2	1.5	0.2	83.5	0.1	9.5
10	500	0.29	—	0.0	0.4	3.8	74.9	1.5	18.4
27	515	0.27	274	0.2	0.1	0.5	90.5	0.1	21.7
Catalyst No. 3									
29	475	0.31	166	0.0	0.4	1.1	89.4	0.4	9.7
30	496	0.29	306	0.0	0.8	0.4	85.3	0.9	17.3
31	515	0.25	382	0.0	0.3	0.9	86.5	0.6	24.7
28	515	0.28	410	0.0	0.1	0.7	84.5	0.9	27.2

It should be remarked that the yield of 27.2% is equivalent to more than 60% conversion of the heptane actually present.

YIELD OF AROMATICS FROM TURNER VALLEY CRUDES BY CATALYTIC CONVERSION

In order to determine the possible yield of aromatics from Turner Valley crudes, catalytic conversion experiments were done on selected cuts from representative Turner Valley crudes. Typical results and calculations are recorded in Tables IV and V.

Effect of Recycling

Experiments in which the product was recycled over the catalyst are recorded in Table VI. In some experiments a small increase in yield of aromatics is indicated, but in others (Expts. 54 to 56) the yield falls off slightly. It appears that, using a catalyst containing 14% chromium trioxide (and the indicated feed rates), a maximum yield is obtained in one pass.

TABLE IV

CATALYTIC PRODUCTION OF AROMATICS FROM SELECTED CUTS FROM TURNER VALLEY CRUDES

Feed: Nitrated toluene cut No. IV. Catalyst No. 1

Origin of sample	Expt. No.	Temp., °C.	Feed rate, cc./gm. catalyst /hr.	Vol. gas, cc./gm. feed	Gas analysis, vol. %					Aromatics, based on original, %
					CO_2	O_2	Unsaturates	H_2	CO	
Royalite No. 45	9	510	0.28	300	0.1	0.7	1.2	83.6	0.5	18.6
Foothills No. 6	12	510	0.31	275	0.4	0.51	1.0	80.0	0.3	15.0
Foundation No. 1	13	510	0.24	330	0.0	0.2	0.8	84.1	0.5	18.8

TABLE V

YIELD OF AROMATICS (BY CONVERSION) FROM ORIGINAL CRUDE

	Royalite No. 45	Foothills No. 6	Foundation No. 1
Volume of crude, cc.	1000	1000	1000
Density of crude	0.8026	0.8193	0.7936
Wt. nitrated toluene cut, gm.	55.1	50.6	82.8
Aromatics from nitrated cut (see Table IV), %	18.6	15.0	18.8
Aromatics, based on crude, by conversion, %	$\frac{18.6 \times 55.1}{802.6} = 1.28$	$\frac{15 \times 50.6}{819.3} = 0.93$	$\frac{18.8 \times 82.8}{793.6} = 1.96$
Toluene, in original crude, %	1.52	1.34	2.05

TABLE VI

AROMATIZATION OF NITRATED TOLUENE CUT (V). TEMPERATURE, 515° C.

Expt.	Feed	State of catalyst	Feed rate, cc./hr. per gm. catalyst	Vol. gas, cc./gm. feed	Aromatics in products, %	Product, as % of feed	Br No.	Aromatics, based on crude, %
Catalyst No. 4								
45	Fresh V	*r**	0.16	326	35.1	84	16.3	2.13
46	Product of 45	*n.r.*†	0.14	277	49.8	85	21.3	2.57
47	Fresh V	*n.r.*	0.07	365	36.0	83	19.1	2.13
48	Fresh V	*r*	0.12	492	41.9	77.6	—	2.35
49	Prod. from 47	*n.r.*	0.14	322	46.4	87	26	2.42
50	Prod. from 49	*r*	0.14	290	60.4	81.5	19.6	2.57
51	Prod. from 50	*n.r.*	0.1	104	68.4	91	20.6	2.65
52	Prod. from 48	*n.r.*	0.17	84	50.3	95.4	20.2	2.69
53	Prod. from 46	*n.r.*	0.09	307	53.9	91	19.5	2.50
Catalyst No. 5								
54	Fresh V	*r*	0.28	385	50.1	75	19.7	2.72
55	Prod. from 54	*n.r.*	0.12	253	55.3	82	14.2	2.46
56	Prod. from 55	*r*	0.12	229	70.9	77	10.8	2.42

*r = Revivified. †n.r. = Not revivified.

Since hydrocarbons such as the dimethylhexanes are present in the nitrated toluene cut, the products of catalytic aromatization will be expected to contain aromatics such as xylene, in addition to toluene.

Composition of Product

Sixty-five cubic centimetres of aromatized product (containing 56.8% aromatics) was fractionated in a small Podbielniak type column. The boiling point curve is recorded in Fig. 2 and indicates that the recovery of toluene from this fraction should not present any great difficulty.

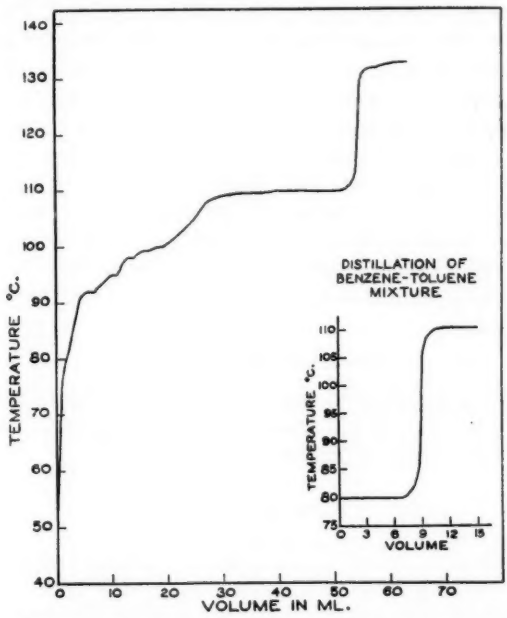

FIG. 2. *Distillation of aromatized cut (pressure, 760 mm.)*

The products of distillation were divided into three cuts: below 90°, 90° to 120°, and above 120° C., and the aromatics determined by dispersion. (The base value for the aromatized material was determined in the usual way and was found to be unchanged.) The results show that the aromatics are 5% benzene, 67% toluene, and 27% xylene. A duplicate experiment on 53 cc. aromatized product showed that the aromatics contained 66% toluene.

Discussion

The foregoing experiments indicate a maximum yield of aromatics of 2.72%, based on the original crude (see Table VI, Expt. 54). Since the aromatics contain 67% toluene the yield of toluene is $2.72 \times 0.67 = 1.82\%$. The original crude contained 1.33% toluene, and thus the total recovery of

toluene would be 3.15%. We may say that aromatization increases the yield of toluene by 137%. Under large-scale operating conditions the yield might possibly be slightly greater than this, since plant losses in handling and evaporation would probably be somewhat less than ours. Supposing a rating of 27,000 bbl. per day (289 lb. per bbl.), the possible recovery of toluene is $\frac{27{,}000 \times 289 \times 3.15 \text{ lb.}}{100} = 250{,}000$ lb. per day for the whole of Turner Valley (1942). The optimum catalyst temperature is 515° C. Experiments using 14.2% chromium trioxide on alumina indicate that the maximum conversion is obtained with one pass over the catalyst. Using 9.7% chromium trioxide on alumina, the yield may be improved by two or three passes.

The optimum rate is approximately 6 gm. feed per hour using 65 gm. catalyst, and corresponds to a contact time of 73 sec. Assuming that the revivifying takes up about as much time as the conversion, about two tons of catalyst would be required per 1000 lb. toluene desired per day. The catalyst actually has quite a long life, but it is inadvisable to use it too long before revivifying since carbon is deposited. When much carbon is deposited, it becomes difficult to avoid overheating the catalyst, with consequent loss in activity, while burning off the carbon. It is suggested that the catalyst be revivified when an amount of feed equal to the weight of the catalyst has been passed over the catalyst.

Acknowledgments

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Note on the Preparation of 1-Methoxy-1,3-butadiene[1]

Recently a method has been described by Meier (2) for the preparation of 1-methoxy-1,3-butadiene by the pyrolysis of 1,1,3-trimethoxybutane over a catalyst of the approximate composition $BaO \cdot 5SiO_2$. However, his description of the preparation of the catalyst is very incomplete and insufficient for the duplication of his results.

A preparation is now described that will produce a catalyst of composition varying from $BaO \cdot 4SiO_2$ to $BaO \cdot 5SiO_2$, with which conversion yields of 40 to 50% can be obtained consistently.

1-Methoxy-1,3-butadiene polymerizes to an appreciable extent on distillation. It could not be converted to a sulphone but it underwent the Diels–Alder reaction with maleic anhydride giving rise to 3-methoxy-1,2,3,6-tetrahydrophthalic anhydride. This last compound, when dehydrogenated, lost the methoxyl group and produced phthalic anhydride.

Experimental

Preparation of the Catalyst.—To a stirred solution of water glass (200 cc., sp. gr. 1.386) in water (500 cc.) was added dropwise a solution of barium nitrate (57.7 gm.) in warm water (500 cc.). Ammonium hydroxide (28%) was then added to the mixture and stirring continued for 20 hr. The resulting gel was collected on a large Büchner funnel, placed in a 4 litre beaker and dialyzed through a non-waterproofed commercial cellophane membrane with stirring and heating (3). The dialysis was continued (six hours) until a test for ammonia with Nessler's reagent was negative. The slurry was allowed to settle and, after decantation of the supernatant water, filtered with suction and dried in an oven at 110° C. Found: BaO, 37.44; SiO_2, 60.41%. Calc. for $BaO \cdot 4SiO_2$: BaO, 38.96; SiO_2, 61.12%. Another batch of catalyst yielded the following figures: BaO, 34.86; SiO_2, 63.06%. The dried catalyst was broken into small pieces of about 0.5 cc.

1,1,3-Trimethoxybutane.—The material was prepared from crotonaldehyde as described by Meier (1). After careful fractionation through a Stedman column, 1,1,3-trimethoxybutane boiled at 55° C. (15 mm.) and 149° to 150° C. (747 mm.), d^{25} 0.916, n_D^{20} 1.4050. Molecular refractivity, found: 39.2; calc., 39.10. Calc. for $C_7H_{16}O_3$: C, 56.76; H, 10.81%; mol. wt. 148. Found: C, 57.57; H, 10.77%; mol. wt. (in benzene, cryosc.) 148.

1-Methoxy-1,3-butadiene.—The catalyst (a volume of about 100 cc.) is loaded into a vertical catalyst tube, 20 mm. inside diameter and 125 cc. capacity, which is inserted inside an electrically heated sleeve of 40 mm. outside diameter. The top part of the catalyst tube is filled with coarse pumice resting on top of the catalyst. The catalyst is activated by heating at 400° C. for 12 hr. in a stream of nitrogen. For operation, a feeding device

[1] *Contribution from the Division of Chemistry, National Research Council, Ottawa, Canada. Issued as N.R.C. No. 1482.*

is connected to the top of the catalyst tube while the lower end is connected to a condensation train cooled in dry-ice–acetone. The catalyst tube is heated, a stream of nitrogen is allowed to flow through the system and, when the required temperature has been reached, the trimethoxybutane is fed in at the desired rate. Trimethoxybutane is vaporized while going through the pumice and mixes with the nitrogen before it reaches the catalyst. The conditions finally adopted involved a temperature of 340° C., a nitrogen flow of 30 litres per hr. and a contact time* of 0.24, which is equivalent to a flow of trimethoxybutane of 0.4 cc. per sec. The catalyst gradually becomes poisoned and the yields decrease accordingly. The yields varied from 40 to 60%, but were most consistently between 40 and 50%.

The product of the pyrolysis was washed with water and fractionated by rapid distillation. An azeotrope of methoxybutadiene and water, b.p. 67° C., was first obtained and then the methoxybutadiene, b.p. 89° to 90° C. Pure 1-methoxy-1,3-butadiene boils at 88° to 89° C. (772 mm.), d^{26} 0.830, n_D^{26} 1.4590. Mol. refractivity found, 27.1; calc., 27.4.

It is interesting to compare the similar constants of 2-methoxy-1,3-butadiene: b.p. 75° C. (745 mm.), n_D^{20} 1.4442, d^{20} 0.8281 (4).

3-Methoxy-1,2,3,6-tetrahydrophthalic Anhydride.—To a stirred solution of 1-methoxy-1,3-butadiene (12 gm.) in dioxane (25 cc.) cooled in an ice-bath was added slowly a solution of maleic anhydride (20 gm.) in dioxane (11 cc.). The reaction mixture was allowed to warm up to room temperature and stand for two hours. Addition of water caused the separation of an oil, which soon solidified. This was filtered, washed with water, and dried. Wt., 7.2 gm. It was found to consist of a large proportion of polymer. The product was digested with boiling benzene and the solution decanted from the insoluble polymer. The decantate was concentrated, allowed to cool, and diluted with petroleum ether. 3-Methoxy-1,2,3,6-tetrahydrophthalic anhydride crystallized out and, after one more crystallization from the same mixture, consisted of clusters of small, colourless, flat prisms, m.p. 99° to 100° C.† Wt., 1 gm. Calc. for $C_9H_{10}O_4$: C, 59.33; H, 5.49; OCH_3, 17.03%. Found: C, 59.12, 59.44; H, 5.57, 5.39; OCH_3, 16.72%.

Dehydrogenation of 3-Methoxy-1,2,3,6-tetrahydrophthalic Anhydride.—3-Methoxy-1,2,3,6-tetrahydrophthalic anhydride was heated with selenium for 30 min. at 325° to 350° C. in a stream of nitrogen, in a distilling flask, the side-arm of which was connected to a cooled receiver. An oil distilled which crystallized in the side-arm and the receiver. This was washed with methanol, the solution evaporated, and the residue fractionated *in vacuo*. A first fraction sublimed at 80° to 90° C. (0.3 mm.) and a second fraction sublimed at 160° to 180° C. (0.3 mm.). Fraction I separated from ether–petroleum-ether as colourless needles, m.p. 134° C., alone or in admixture with phthalic anhydride. The second fraction, crystallized from ethyl acetate, consisted

* *The contact time was calculated as the volume of trimethoxybutane in cubic centimetres of liquid per volume of catalyst per hour.*

† *All melting points are corrected.*

of small, colourless prisms, m.p. 214° C. In admixture with phthalic acid (m.p. 215° C.) it melted at 214.5° C. Calc. for $C_8H_6O_4$: C, 57.83; H, 3.61%. Found: C, 57.76; H, 3.75%.

Dehydrogenation by means of *n*-amyl disulphide (5), which does not involve as high a temperature, caused demethoxylation and gave rise to phthalic anhydride.

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